

# Biosynthesis of Cell Walls of Fungi

V. FARKAŠ

*Institute of Chemistry, Department of Biochemistry of Saccharides, Slovak Academy of Sciences, 809 33  
Bratislava, Czechoslovakia*

INTRODUCTION	117
FUNGAL CELL WALLS	117
Chemical Structure	117
Wall Architecture	118
FORMATION OF CELL WALLS	120
General	120
Biosynthesis of Individual Wall Components	121
Chitin	121
Glucans	124
Polysaccharide-protein complexes	126
(i) Biosynthesis	126
(ii) Regulation	128
(iii) Localization of reactions	129
Formation of Wall Fabric	129
Metabolic Stability of the Wall	130
MORPHOGENETIC ASPECTS	131
Topology of Wall Growth	131
Speculations About the Possible Regulatory Mechanism	133
SUMMARY AND CONCLUSIONS	135
LITERATURE CITED	135

## INTRODUCTION

The surface structure of fungi, the cell wall, with all its specialized functions in the diverse life activities of fungal cells, increasingly attracts the attention not only of mycologists but also of the workers of other biological disciplines. The fungal cell walls have been studied from different aspects, and many excellent reviews have been written in the past (e.g., 5, 9, 15-17, 23, 46, 91, 205, 213).

Papers dealing with the molecular mechanism of cell wall formation in fungi started to appear with increased frequency after the discovery of nucleoside diphosphate-sugars and their function as glycosyl donors in the biosynthesis of complex saccharides (156) and the commercial availability of these compounds labeled with radioactive nuclides. Whereas in 1965, at the time of Aronson's review (5) on fungal cell walls, only a few (1, 98) papers dealing with the "in vitro" biosynthesis of fungal wall components had been published, the present complete bibliography in this field would mount up to hundreds of papers.

This by itself indicates the increasing importance of studies on the mechanism of biosynthesis of cell surface structures, using the fungi as models for simple eucaryotic cells. The rationale for these studies is that they could not only explain the mechanism of biosynthesis of

different cell wall components but also contribute to a better understanding of various surface-related biological phenomena, such as cell-cell interactions, immune response, morphogenesis, drug resistance, and others.

Because of the great complexity of these problems, I will deal in this review mainly with the molecular aspects of cell wall formation in fungi and try to find their possible relationship to regulation of morphological development.

## FUNGAL CELL WALLS

Detailed discussion about the structure and chemical composition of fungal cell walls falls outside the scope of this review; besides, there have been several comprehensive articles written on this subject in the last years (e.g., 5, 9, 10, 15, 16, 205). However, some general explanations are necessary to serve as an introduction to the problem of fungal cell walls.

### Chemical Structure

The remarkable properties of fungal walls, such as their mechanical strength, morphological features, and biological activity, are undoubtedly based on their particular chemical composition. Bartnicki-Garcia (15) pointed out that a close correlation exists between taxonomic classification and cell wall composition among fungi.

Polysaccharides, which represent about 80 to

90% of the dry matter of fungal cell walls, are composed of amino sugars, hexoses, hexuronic acids, methylpentoses, and pentoses (16). Glucose and *N*-acetyl-D-glucosamine (GlcNAc) usually represent the chemical elements of skeletal wall polysaccharides, such as chitin, cellulose, noncellulosic  $\beta$ -glucans, and  $\alpha$ -glucans. The other sugars are present mainly in the form of various homo- and heteropolysaccharides, often in chemical complexes with proteins.

It can be anticipated that, owing to distinctive physicochemical properties, the different polysaccharides fulfill specific functions in the cell walls. Whereas the crystalline polysaccharides chitin and  $\beta$ -glucans are the components responsible for the mechanical strength of the wall, the amorphous homo- and heteropolysaccharides, often in association with proteins, play the role of cementing substances and constitute the carbohydrate moieties of extracellular enzymes and cell wall antigens (10, 91, 146).

### Wall Architecture

The functional specialization of each cell wall component is reflected also in its location within the wall structure. Electron microscopic and cytochemical evidence (5, 58, 65, 121, 161, 209, 210, 266, 267), combined with results of chemical and enzymatic analyses (118, 119, 135, 146, 185, 200, 255; M. Kopecká, H. J. Phaff, and G. H. Fleet, Proc. Int. Symp. Yeasts 4th, Vienna, Austria, 1974, D5, p. 205), indicate that a certain degree of stratification exists in the walls of fungi. Although distinct layering of building material is rarely seen on ultrathin sections through the cell walls, the general picture is that the outer surfaces of the wall are smooth or slightly granular in texture and composed of amorphous material (often glycoprotein in nature), whereas the skeletal microcrystalline component is prominent in the layer of the wall adjacent to plasmalemma. The interfibrillar spaces of the inner wall layer are filled with amorphous material, probably of the same chemical composition as that of the outer wall layer. The amorphous matrix material penetrates also into the periplasmic space (e.g., 86, 161, 242, 266, 267), where some of its constituents may exhibit different enzymatic activities (10, 146).

The overall appearance of fungal walls varies with age (Fig. 1). Newly synthesized portions of the walls are thin and smooth, with no visible stratification. In older portions the primary wall is covered with secondary layers composed of amorphous matrix material, and the fibrillar texture of the innermost wall layer becomes more pronounced (118, 120, 121, 253, 254).

There has been some discussion in the litera-

ture about the possible artifactual nature of the microfibrillar cell wall components (37, 172). The main argument against the presence of distinct layers of building material in the fungal cell walls was the possibility that the polysaccharide microfibrils might arise as a consequence of various chemical treatments of isolated cell walls (7, 116). These assumptions seemed to be substantiated to a certain degree by Eddy and Woodhead (71), who observed "in vitro" reaggregation of fibrillar elements of alkali-dissolved yeast wall glucan.

Nevertheless, the presence of microfibrils of skeletal wall polysaccharides can almost invariably be demonstrated in cell walls formed de novo on the surfaces of regenerating fungal protoplasts (e.g., 2, 88, 96, 197, 247, 265). Also, refined cytological and biochemical methods, such as partial enzymatic digestion of the walls followed by electron microscopy, reveal the fibrous nature of certain wall components (118, 119, 121, 135; Kopecká et al., Proc. Int. Symp. Yeasts 4th, Vienna, Austria, 1974, D5, p. 205).

It should be noted, however, that observations made by application of the enzyme dissection technique to reveal wall architecture do not allow straightforward interpretation in each case and should be treated cautiously. Several factors may influence the final conclusions. (i) The cross-linking between the individual wall components may result in the removal of the enzymatically solubilized polymer as well as the second, linked polymer. (ii) The lytic enzymes used may be insufficiently purified—even traces of contaminating hydrolytic enzyme activities can substantially influence the final image; equally important is knowledge on the precise modes of action of the enzymes used. (iii) The partial dissolution of the wall by enzymic or chemical treatments may lead to rearrangement and displacement of the remaining wall constituents, thus changing the obtained electron microscopic image. (iv) Different accessibilities of the individual cell wall components to enzyme attack when working with intact cells or the isolated walls: in isolated walls the applied enzymes can attack the wall structure from both sides, whereas in intact cells only the components located on the outer wall surface are susceptible.

The methodological difficulties described above are perhaps the main sources of controversial conclusions concerning the details of wall architecture in fungi. This situation can be best demonstrated by the existing uncertainty about the structure of the cell wall in *Saccharomyces* sp., so far the best studied fungal cell envelope.

Based on the available chemical, biochemical, immunochemical, and cytological evidence, Lampen (146) suggested a structural model for

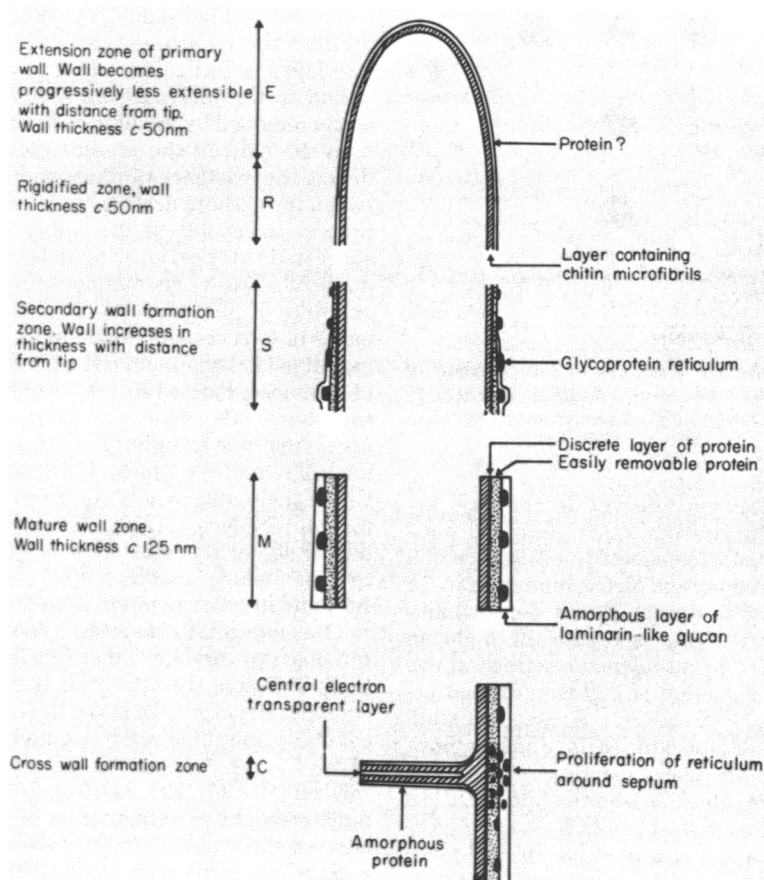


FIG. 1. Wall and septal structure of a hypha of *Neurospora crassa*. (Reproduced with permission from reference 253.)

the cell wall of *Saccharomyces cerevisiae*. In his model the innermost microfibrillar layer, composed of insoluble  $\beta$ -glucan, is linked via protein to the outer wall layer, composed of mannan-protein molecules mutually linked by phosphodiester bridges between their polysaccharide moieties. The phosphodiester cross-linking was supposed to form a physical barrier that holds within the wall structure the extracellular mannan-protein enzymes invertase, acid phosphatase, and others. The latter conclusion was arrived at from the effect of the so-called PR-factor, a phosphomannanase partially purified from culture filtrates of *Bacillus circulans* (180). Treatment of intact cells with this preparation liberated the invertase and large phosphomannan molecules and was followed by complete dissolution of the cell wall. The PR-factor supposedly cleaved selectively the phosphodiester links between the mannan molecules.

The observation that invertase can be liberated from intact yeast cells by mild sonication

or treatment with thiol reagents led Kidby and Davies (134) to a conclude that invertase is not chemically bound to the cell wall and that the natural barrier against its escape from the wall structure is the external wall layer, composed of mannan-protein molecules linked together, as suggested by Lampen (146), by phosphodiester bridges but also by disulfide linkages between their protein moieties (Fig. 2).

Apparently contradictory results were obtained by Arnold (4), who demonstrated that in ethyl acetate-treated cells the sulfhydryl compounds on their own were ineffective in releasing the invertase entrapped within the wall; they did, however, increase the extractability of the marker enzyme by yeast extracts containing glucan-degrading activity. The differences as to extractability of invertase by thiol reagents, when compared with results of Kidby and Davies (134), could be explained by possible irreversible changes in the upper cell wall layer caused by the ethyl acetate treatment.

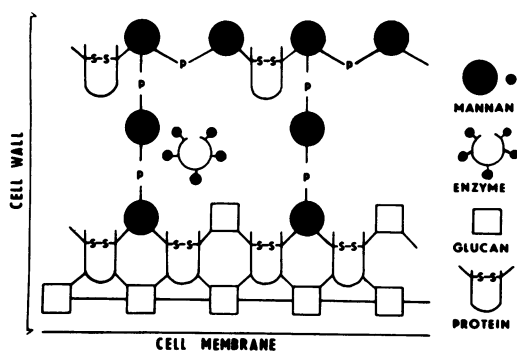


FIG. 2. Hypothetical structure of the cell wall of the yeast *S. cerevisiae*. S—S, Disulfide linkage; P, phosphodiester bridge. (Modified from Kidby and Davies [134].)

The difficulties encountered in chemical extraction of  $\beta$ -glucan from intact yeast cell walls led Bacon et al. (7) to suggest the existence of a thin chitinous membrane on the outer surface of the cell walls that would form a permeability barrier preventing the extraction of  $\beta$ -glucan with the alkali. The problems described above with alkaline extraction of  $\beta$ -glucan did not occur with isolated cell walls (7). Subsequent studies from different laboratories have shown, however, that the exclusive location of chitin in *Saccharomyces* cell walls is in the bud scars (48, 229).

Cytochemical staining selective for acid phosphatase (161) has revealed that this extracellular mannoprotein enzyme is located both in the outer wall layer and in the innermost layer, penetrating into the periplasmic space. The localization of mannan-proteins in the outer and the innermost regions of the yeast walls has been demonstrated also by other cytochemical methods (58, 210, 266, 267).

The periplasmic space of yeasts has been also reported to contain large amounts of glycogen (105, 106); its role, however, is still obscure.

The presence of mannoprotein complexes in the upper wall layer in yeast is well supported by the fact that synergistic action of proteases and  $\beta$ -glucanases is required to dissolve completely the walls of the intact cells (87, 214, 264, 268, 269). The effect of proteases can be replaced by thiol reagents but not by the action of purified bacterial  $\alpha$ -mannanase; neither is the  $\alpha$ -mannanase alone effective in substantial removal of the mannan located at the wall surface (268, 269). These results indicate that proteins held together by disulfide bridges form a protective barrier against the penetration of exogenous glucanase. On the other hand, walls isolated from mechanically disintegrated cells are readily dis-

solved by purified endo-1,3- $\beta$ -glucanase, indicating that the innermost, periplasmic mannoprotein layer is, unlike the outer one, only loosely bound to the internal wall surface and can be easily removed by washing with water (268, 269).

In the light of the present experimental evidence, the existence of phosphodiester links between the carbohydrate moieties of the mannan-protein molecules in the upper wall layer, as suggested in previous models (134, 146), is doubtful. The yeast mannans contain variable amounts of phosphate (9, 10), which, however, exists in *S. cerevisiae* mannan as a diester linking an  $\alpha$ -(1,3)-mannobiosyl unit to position C-6 of mannose, located in the terminal position of the short side chain (10, 250). In *Kloeckera brevis* mannan the phosphorus is present in the form of mannosyl-1-phosphate linked to position C-6 of the mannose unit in the side chain of the mannan molecule (250). However, direct evidence about the existence of phosphodiester bridges linking together two macromolecular subunits in yeast mannan is so far lacking.

The penetration of certain types of large macromolecules through the cell wall in both directions indicates that the wall is by no means a compact structure. To allow the release of intracellularly manufactured enzymes and the passage of certain exogenous macromolecules into the cell (124, 201, 222, 223, 248, 276), the cell wall must possess a certain number of pores. At present, it is difficult to decide whether the pores are a permanent structure within the wall and to determine their location. An alternate explanation, offered by Scherrer et al. (222), is that the physicochemical interactions of penetrating macromolecules with the components of the cell wall may evoke a reorganization within the wall structure resulting in the tentative formation of openings large enough to accommodate the penetrating macromolecules.

## FORMATION OF CELL WALLS

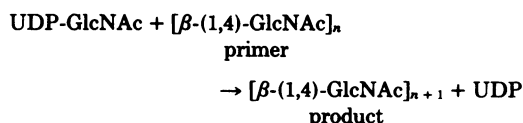
### General

The problems connected with the process of cell wall formation in fungi are being currently investigated from different points of view. Among the most frequently studied questions are: (i) the molecular mechanisms of the biosynthesis of individual cell wall components, (ii) the involvement of cellular structures in these processes and sites of participating enzyme activities, (iii) the participation of lytic enzymes in the process of cell wall formation, (iv) regulatory aspects at different levels, and (v) cell wall formation in relation to morphological development of the cells.

Accordingly, different experimental systems have been used in these studies, ranging in degree of complexity from isolated enzymes or enzyme systems through subcellular fractions and protoplasts to intact cells. The experimental methods vary from purely chemical and biochemical to cytological and physical. In the following sections I will briefly review the present knowledge on the process of cell wall formation in fungi, starting from the biosynthesis of the individual principal wall polymers.

### Biosynthesis of Individual Wall Components

**Chitin.** Chitin, a  $\beta$ -(1,4) polymer of GlcNAc, is the major cell wall component in most filamentous fungi. Its *in vitro* biosynthesis in a cell-free system was first described by Glaser and Brown (98), who found that an enzyme preparation from *Neurospora crassa* catalyzes the incorporation of GlcNAc units from uridine 5'-diphosphate (UDP)-GlcNAc into a polymer undistinguishable from the authentic chitin. The general equation for this reaction is:



The reaction requires the presence of divalent cations and GlcNAc as activators. A single enzyme, UDP-2-acetamido-2-deoxy-D-glucose:chitin 4- $\beta$ -acetamidodeoxyglucosyltransferase (EC 2.4.1.16), known under the trivial name chitin synthase, seems to be involved in the reaction. Particulate preparations of chitin synthase have been prepared from a wide range of fungi (e.g., 3, 39, 55, 76, 101, 102, 123, 133, 164, 177, 181, 195, 196, 219, 220, 260), and most of them have similar kinetic properties (101). The results obtained with different enzyme preparations indicate that chitin synthase is an allosteric enzyme, having more than one binding site per molecule (55, 63, 181). In the absence of GlcNAc the Hill numbers are close to 4 at low substrate concentrations (below 0.1 mM), and values close to 2 at higher substrate concentrations are obtained (63, 101). In the presence of GlcNAc the Hill number is close to 1 (219). The transfer of GlcNAc units from UDP-GlcNAc to a primer molecule seems to proceed in a single step, and there is, so far, no evidence about the participation of "lipid intermediate" in this reaction (77, 183).

The presence of an endogenous primer is not absolutely necessary for the reaction to proceed. Solubilized preparations of chitin synthase are capable of forming chitin under conditions when

evidently no macromolecular primer is present (98, 101, 102, 215).

GlcNAc appears to act as a positive allosteric effector of particulate chitin synthase (55, 181, 216, 219). However, since relatively high concentrations of a GlcNAc are needed to produce the same activation as that produced by much lower concentrations of UDP-GlcNAc, it has been suggested that UDP-GlcNAc is the natural effector and that GlcNAc simply mimics its effect (216). The possibility has been considered that an additional stimulatory effect of GlcNAc on chitin biosynthesis *in vitro* might reside in its function as the primer for initiation of new chains of chitin (55, 183); however, this has found very little experimental support (102, 216). Interestingly, *N,N'*-diacetylchitobiose but higher *N*-acetylchitodextrins especially stimulate the *in vitro* biosynthesis of chitin both in the absence and in the presence of GlcNAc (183, 204, 208). On the other hand, the activity of enzymes solubilized with butanol (98) or digitonin (63) is not affected by GlcNAc.

One of the most powerful inhibitors of fungal chitin synthase is the pyrimidine antibiotic polyoxin D (e.g., 20, 77, 101, 102). The inhibition by polyoxin D is competitive with regard to the substrate UDP-GlcNAc, the inhibitor constant,  $K_i$ , being two to three orders of magnitude lower than the Michaelis constant,  $K_m$ , for the substrate (20, 76, 103, 133, 164). A mechanism has been suggested whereby polyoxin D, owing to its structural similarity with UDP-GlcNAc, competes for the active site of the enzyme (115).

Fungal chitin synthase is normally prepared as a particulate enzyme associated with cellular membranes. Numerous studies with cell-free extracts from various fungi have shown that the highest specific activity of chitin synthase is present in a membrane fraction vaguely termed "microsomal" (3, 101, 103, 164, 188, 204). The particular membrane to which chitin synthase is attached was, until recently, difficult to identify. One of the factors negatively influencing the separation of membrane fractions might be that mechanical disintegration of the cells before enzyme extraction as well as bursting of protoplasts by osmotic shock could cause fragmentation of cellular membranes and their vesiculation (68).

Using concanavalin A to preserve the integrity of the plasmalemma, Durán et al. (69) were able to show that yeast chitin synthase is located almost exclusively in the plasmalemma. Similarly, isopycnic density gradient centrifugation of membrane preparations from *Phycomyces blakesleeana* (123) or *Candida albicans* (39) points out that the plasmalemma is the principal site of chitin biosynthesis.

Nevertheless, there have been repeated claims (183, 186, 204) that the bulk of chitin synthase is located directly in the cell wall. It is highly probable that in these cases the wall preparations were contaminated with the remnants of cellular membranes, especially plasmalemma, as it has been demonstrated with cell walls prepared from *S. cerevisiae* by mechanical disintegration of the cells (245). The fact that fungal protoplasts can be effectively used for preparation of the enzyme (3, 133, 188) is also against predominant location of chitin synthase in the cell walls. As will be shown later, the location of chitin synthase, and possibly also of some other polysaccharide synthases, in the plasmalemma could be of essential importance for regulation and spatial organization of cell wall synthesis in fungi.

The membrane-bound chitin synthase can be liberated by butanol extraction (98), by digitonin treatment (63, 70, 101), or by incubation of the particulate enzyme with the substrate and activator (215). Whereas both butanol and digitonin destroy the basic membrane structure, the solubilizing effect of UDP-GlcNAc together with GlcNAc is difficult to explain.

Ruiz-Herrera and Bartnicki-Garcia (215) postulated a working hypothesis according to which the chitin synthase is released from the membrane structure as soon as it starts the synthesis of chitin. If it were so, the presence of chitin synthase in the cell wall would not be surprising. However, the mechanism by which the substrate for continuous chitin synthesis would be effectively supplied from the cell to the cell wall is difficult to envisage. In later experiments of the same authors and co-workers (215, 216, 218), it was established that the "soluble" preparation of chitin synthase consisted in fact of particles termed "chitosomes."

The molecular weight of chitin synthase from *Coprinus cinereus* solubilized by digitonin treatment is of several millions (63); however, in the presence of high salt concentrations the aggregates dissociate to smaller active subunits with molecular weights of about 150,000 (101).

Preparations of chitin synthase solubilized from membranes of *Mucor rouxii* by incubation with the substrate and activator (215) or isolated from the cytoplasm of mechanically disintegrated cells (216) contain enzyme granules of about 35 to 100 nm in diameter. On incubation with UDP-GlcNAc, chitin microfibrils in association with chitin synthase granules are produced (38, 216). The terminal position of enzyme granules on the microfibrils formed indicates that the latter are elaborated by end synthesis of a large number of parallel chitin chains rather

than by spontaneous random crystallization of de novo-synthesized chitin molecules (Fig. 3).

Ultrastructural studies revealed a rather complicated structure for chitin synthase particles, or, as they have been later renamed, chitosomes (38). Freshly isolated chitosomes appear on ultrathin sections as clusters of protein granules bound within a membranous shell. During fibrillogenesis the chitosomes undergo a series of ultrastructural changes: the protein granules disappear, and, instead, a coiled microfibril of chitin appears inside the chitosome; the shell of the chitosome opens, and the chitin microfibril extends from the particle (218). More recently (18), functional chitosomes have been isolated from a series of different genera of fungi, including yeasts.

Although the chitosomes are fully capable of synthesizing chitin microfibrils in vitro, their existence in vivo has not yet been fully confirmed. With regard to their relatively large dimensions, they cannot be considered as integral components of the plasmalemma or another cellular membrane (average membrane thickness is 8 to 9 nm). They could possibly represent containers of chitin synthase conveying the enzyme from the site of its synthesis to its destination at the cell surface, or, eventually, they could be of artifactual nature. The latter possibility is indicated by the presence of intracellular enzymes and, in some cases, of ribosomes in the isolated chitosomes (216). The artifactual nature of chitosomes is further indicated by the fact that molecules of chitin synthase solubilized from the cell walls of *M. rouxii* by digitonin treatment associate with one another to form vesiculoid structures morphologically and functionally resembling chitosomes (S. Bartnicki-Garcia, C. E. Bracker, and J. Ruiz-Herrera, Abstr. Int. Mycol. Congr. 2nd, Tampa, Fla., p. 41, 1977).

Perhaps the most remarkable property of fungal chitin synthase is that it exists in cells largely in an inactive, or zymogenic, state. The inactive enzyme can be converted to the active form by limited proteolysis. The phenomenon of proteolytic activation was first discovered with yeast chitin synthase (52) and later confirmed also with preparations from other sources (3, 50, 102, 107, 122, 164, 182, 215, 219, 220, 260). The zymogenic character of chitin synthase was confirmed also in solubilized preparations (70, 215).

Crude preparations of yeast chitin synthase obtained from protoplast lysates underwent a slow but significant increase in specific activity upon standing or storage at low temperatures, indicating that they might contain some intrinsic factor capable of activating chitin synthase. Mild

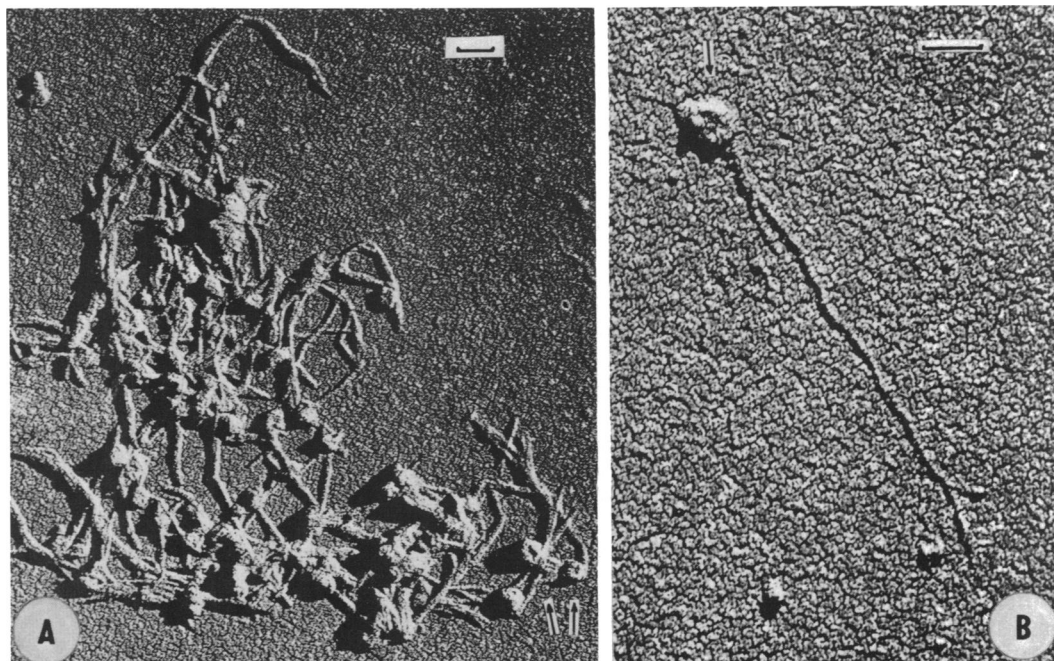


FIG. 3. Electron micrographs of isolated chitosomes from *M. rouxii* incubated with substrate. Chitosome granules are marked with arrows. (A) Group of chitosomes; (B) isolated chitosome with extending chitin microfibril. (Reproduced with permission from reference 218.) Bar represents 0.1  $\mu\text{m}$ .

sonication of the pellets from the protoplast lystate led to solubilization of the so-called activating factor whereby chitin synthase remained insoluble (50). The chitin synthase freed from the activating factor had only negligible activity unless it was preincubated with the activating factor or with trypsin. Subsequent studies have shown that the activating factor is a protease (54) located in some kind of intracytoplasmic vesicle, unseparable from the vacuolar fraction (53). The properties of purified activating factor were identical with those described for yeast proteinase B (108, 259), known to be functional in inactivating yeast tryptophan synthase (109, 221). Other yeast proteases are unable to activate chitin synthase, although many proteases from other sources, for example, trypsin, chymotrypsin, papain, subtilisin, rennilase, and acid proteinase, are capable of doing so; however, the activating effects of the individual proteases are not the same in all cases (164).

The supernatant fraction obtained after lysis of yeast protoplasts has been found to contain a heat-stable protein capable of selective binding to the activating factor, thus rendering it ineffective (50). The inhibitor of the activating factor was purified to homogeneity and found to be a low-molecular-weight peptide (molecular weight, 8,500) lacking cysteine, methionine, ar-

ginine, and tryptophan (258). López-Romero et al. (163) isolated from extracts of *M. rouxii* another protein capable of inhibiting the *in vitro* synthesis of chitin. In contrast to the inhibitor from *S. cerevisiae*, the isolated substance did not interfere with the proteolytic activation of zymogen by the activating factor, but it directly inhibited the preactivated chitin synthase.

The discovery of the activating factor and of its specific inhibitor led to an assumption that they might be the components of the system regulating yeast chitin synthase activity *in vivo*. The exclusive location of chitin in yeast bud scars (48, 49, 229) and the observation that its synthesis is restricted to a limited portion of the cell cycle (50, 111) strongly indicated that such a regulatory mechanism could exist. Based on the described properties of the individual components of the system catalyzing the formation of chitin, a working hypothesis has been proposed by Cabib and co-workers (47, 51) for the mechanism of regulation of chitin synthase activity in yeast (Fig. 4).

How this mechanism might operate *in vivo* remains still unclear. The idea is that the vesicles carrying the activating factor would coalesce with the plasmalemma at the site of bud formation, fuse with it, and activate the chitin synthase zymogen at the given site (50). The



particulate enzyme preparations could hydrolyze the product or substrate or both in the course of the enzyme assay (195).

Nevertheless, several cases have been reported in the literature demonstrating *in vitro* biosynthesis of wall glucan. Wang and Bartnicki-Garcia (270) isolated from mechanically disintegrated cells of *Phytophthora cinnamomi* a particulate fraction catalyzing the incorporation of radioactive glucose from UDP- $^{14}\text{C}$ glucose into a glucose polymer containing both  $\beta$ -1,3- and  $\beta$ -1,6-glycosidic bonds. The highest specific activity of glucan synthase was found in the fraction enriched in cell walls. The small yields of the product and the complexity of the enzyme preparation used precluded better characterization of the product. In more recent experiments the same authors (272) took precautions to minimize the formation of proteases in the growing fungus by increasing the concentration of glucose in the growth medium. The mixed membrane fraction (devoid of cell walls) prepared from such cells catalyzed massive formation of  $\beta$ -glucan microfibrils from UDP-glucose. Interestingly, the treatment of the enzyme preparation with trypsin caused a nearly twofold increase of glucan synthase activity, indicating that at least a part of the enzyme was present in zymogenic form or was hindered from the access of the substrate (272). Disaccharides, such as cellobiose, stimulated the synthesis of glucan in the cell-free system. The product was characterized on the basis of its digestibility with purified fungal exo-1,3- $\beta$ -glucanase and X-ray diffraction. The results showed the absence of  $\beta$ -1,6-glycosidic linkages in the product, indicating that it consists entirely of  $\beta$ -1,3-glucan chains (272).

Using cellular homogenates of *Cochliobolus miyabeanus*, Namba and Kuroda (195, 196) demonstrated incorporation of  $^{14}\text{C}$ glucose from UDP- $^{14}\text{C}$ glucose into a polysaccharide precipitable with ethanol. Chemical and structural analysis of the reaction product showed the presence of both  $\beta$ -1,3- and  $\beta$ -1,6-glycosidic linkages between the individual glucose units (196).

In whole cells of *S. cerevisiae* made permeable with toluene-ethanol treatment, Sentandreu et al. (231) observed incorporation of labeled glucose from UDP- $^{14}\text{C}$ glucose into  $\beta$ -1,3-glucan. The radioactive glucan was recovered from the cell homogenate in a membrane fraction by means of differential centrifugation. However, when the membrane particles themselves were assayed for glucan synthase activity, none was detected (231). Attempts to detect the participation of a lipid intermediate in the described reaction have failed.

Bálint et al. (8) prepared from the yeast *S. cerevisiae* a particulate enzyme system composed of mixed membrane fraction catalyzing the formation of  $\beta$ -glucans from both UDP- $^{14}\text{C}$ glucose and guanosine 5'-diphosphate (GDP)- $^{14}\text{C}$ glucose. Practically no activity was found in the wall fraction. The enzyme preparation appeared to contain two independent glucosyltransferase activities, one using UDP-glucose and the other one using GDP-glucose as the respective substrates. A great portion of the radioactivity incorporated was liberated from the particles by mild alkali treatment, indicating that at least a part of the synthesized glucans were of low molecular weight, possibly attached to particles via bonds to protein. Digestion of the respective products with different, partially purified  $\beta$ -glucanases indicated that the product from UDP-glucose contained a higher proportion of  $\beta$ -1,3 bonds whereas the product from GDP-glucose was more abundant in  $\beta$ -1,6 bonds (8).

In a similar system López-Romero and Ruiz-Herrera (165) detected the formation of mixed  $\beta$ -1,3- and  $\beta$ -1,6-glucan from UDP-glucose as the glucosyl donor, whereas the amount of radioactivity incorporated from GDP-glucose was negligible. Contrary to the findings of Bálint et al. (8), the highest specific activity of glucan synthase was found in the cell wall fraction.

Biosynthesis of  $\beta$ -glucan from UDP-glucose was described in less detail also in the *Neurospora* system (186), where glucan-synthesizing activity was confined mostly to the cell walls.

Fèvre and Dumas (84) demonstrated the biosynthesis of polysaccharides of the cellulose type by cell-free extracts from *Saprolegnia monoica*, using UDP-glucose as the substrate. The reaction was stimulated by the presence of  $\text{Mg}^{2+}$  and cellobiose. High substrate concentrations increased the proportion of alkali-insoluble glucans formed in the reaction mixture.

The highest specific activities of glucan-synthesizing enzymes have been found in cell wall and microsomal fractions, and they have been higher in branched hyphae than in unbranched mycelia. Practically no glucan synthase activity has been found in cytoplasm; however, the latter contained a thermolabile component capable of inactivating particulate glucan synthase.

From the results described above, it is apparent that also in the case of glucan biosynthesis uncertainty exists concerning the cellular location of glucan synthase. The physicochemical properties of  $\beta$ -glucan, namely, its insolubility and high degree of crystallinity, indicate that its synthesis occurs most probably *in situ*, i.e., in the cell wall or at the outer surface of plasma-

lemma. The extracytoplasmic location of glucan-synthesizing enzyme(s) is also supported to a certain degree by the observation that germinating cysts of *Phytophthora* incorporated radioactivity from exogenously added UDP- $^{14}\text{C}$ -glucose into their walls (178). No proof, however, was presented to exclude the possibility that added UDP- $^{14}\text{C}$ -glucose was split by extracellular hydrolases, thus enabling the permeation of radioactive glucose into the cell. Clearly, the problem of cellular location of glucan synthase (as well as of other polysaccharide synthases) requires further careful examination.

**Polysaccharide-protein complexes.** Polysaccharides covalently linked to protein represent in some fungi, but especially in yeasts, a principal cell wall constituent (15, 205). Structurally and functionally, the fungal glycoproteins show many similarities with the protein-polysaccharide complexes of higher organisms. For this reason, the fungal glycoproteins can be considered as very appropriate models to study the general aspects of glycoprotein structure, function, and biosynthesis.

The most systematic study on fungal glycoproteins has, so far, been performed with yeast mannan. Recent reviews by Ballou (9) and Cabib (46) comprehensively summarize from different points of view the present knowledge on this subject. Nevertheless, for the sake of completeness I will briefly review the older facts and try to give a more updated version of the achievements in this area.

Yeast mannan is a polymer composed of protein and two carbohydrate moieties differing in their structure and mode of attachment to the peptide (Fig. 6). The polysaccharide moiety is represented by an  $\alpha$ -1,6-linked polymannose backbone to which short chains of mannosyl units linked together by  $\alpha$ -1,2- and  $\alpha$ -1,3-glycosidic bonds are attached predominantly by means of an  $\alpha$ -1,2 bond. The polysaccharide moiety is linked via a diacetylchitobiose bridge by an *N*-glycosidic bond to an asparaginyl residue in the protein part of the molecule (191). The second carbohydrate moiety of yeast mannan consists of short mannooligosaccharides containing both  $\alpha$ -1,2- and  $\alpha$ -1,3-glycosidic bonds attached at their reducing ends by an *O*-glycosidic linkage to serine or threonine residues or both in the protein, from which they can be liberated by  $\beta$ -elimination in weak alkali (190, 234, 235). A species of mannoprotein containing only the *O*-glycosidically linked carbohydrate moiety has been described in *Hansenula wingei* (275).

The biosynthesis of mannan has been investigated from different aspects, such as the mech-

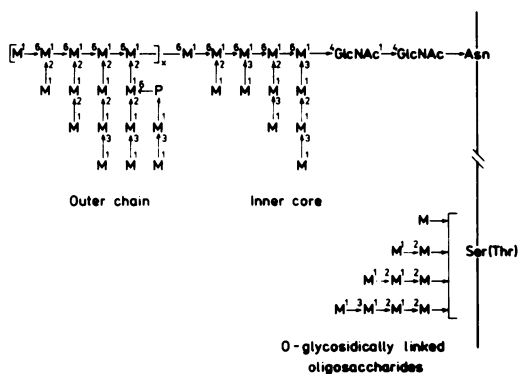


FIG. 6. Structure of yeast mannan. M, Mannopyranose residue; P, phosphate; Asn, asparagine; Ser, serine; Thr, threonine. (From Nakajima and Ballou [191].)

anism of polymerization of mannosyl units, cellular location of individual reactions, mode of transport of mannoproteins from the site of synthesis to the cell wall, and others.

**(i) Biosynthesis.** The structural complexity of the yeast mannan molecule suggests that its formation would require participation of a multienzyme system consisting of different glycosyltransferases. It has been estimated (9) that at least 10 mannosyltransferases, each of them catalyzing the formation of a specific glycosidic bond, are involved in the biosynthesis of carbohydrate moieties of the mannoprotein molecule.

All mannosyl units in the mannan molecule originate from GDP-mannose as the mannosyl donor (1, 24). In some cases, however, the transfer of mannosyls to mannan does not occur directly but through intermediates of lipophilic nature (233, 249). The intermediates have been isolated and characterized as belonging to the group of dolichol phosphates containing 16 to 18 polyisoprene units in their molecules (127, 233).

The participation of dolichol phosphates seems to be undoubted, especially in transfer of those glycosyl units that are directly linked to protein. For example, the attachment of the first mannosyl unit to serine or threonine by formation of an *O*-glycosidic bond to the peptide involves a two-step mechanism in which the mannosyl unit from GDP-mannose is first transferred to dolichol phosphate and, in the second step, transferred from the formed dolichol monophosphate-mannose to the protein acceptor according to the following sequence (6, 236): (i) GDP-mannose + dolichol phosphate  $\rightarrow$  dolichol phosphate-mannose + GDP; (ii) dolichol phosphate-mannose + protein (serine or threonine)  $\rightarrow$  protein (serine or threonine)-mannose + dolichol phosphate. The mannosyl unit, once attached in

this way to protein, can be further mannosylated without involvement of dolichol phosphate (6).

The formation of the *N*-glycosidically linked inner core involves a similar, but more complicated, reaction sequence. The first step in this process is the formation of dolichol pyrophosphate-GlcNAc from dolichol monophosphate and UDP-GlcNAc (152). By a sequence of further transglycosyl reactions, another unit of GlcNAc is added, and the formed dolichol pyrophosphate-*N,N'*-diacetylchitobiose is further mannosylated by one mannosyl unit attached by a  $\beta$ -1,3 bond and by one or more mannosyl units mutually linked by  $\alpha$ -glycosidic bonds (152, 154, 194, 202, 203). There is preliminary evidence (67; W. Tanner, personal communication) to suggest that the subsequent mannosylation of the lipid-linked *N,N'*-diacetylchitobiose proceeds, at least partly, with involvement of dolichol monophosphate-mannose as the immediate mannosyl donor. In a final step the lipid-bound oligosaccharide is transferred to a protein acceptor with formation of an *N*-glycosidic bond (Fig. 7).

From the experiments *in vitro* it is difficult to ascertain what size the lipid-bound oligosaccharide can attain before it is transferred to protein. The smallest transferable unit seems to be the *N,N'*-diacetylchitobiose (155, 194), but the size of lipid-bound oligosaccharide can reach over 12 hexose units (154, 202, 203). Thus, it seems very likely that the formation of the whole inner core of the polysaccharide moiety in the mannan molecule, comprising about 12 to 17 mannosyl units attached to *N,N'*-diacetylchitobiose (192), can take place on the lipid. The reaction sequence of biosynthesis of the *N*-asparaginyl-linked carbohydrate moiety in yeast mannan

strikingly resembles the situation found in various mammalian (25, 59, 117, 157) and plant (149) systems.

Although the syntheses of both carbohydrate moieties in the molecule of yeast mannan proceed independently (82), it is not known whether the same dolichol monophosphate is used for transfer of mannosyl units to both the polymannose portion and the *O*-glycosidically linked oligosaccharides. That this could be indicated by the observation that in liver microsomes (262) the same dolichol phosphate serves for transfers of mannose, glucose, and *N*-acetylglucosamine from their respective nucleoside diphosphates to endogenous acceptors.

Once linkage of the inner core oligosaccharide to the peptide acceptor has been established, the further elongation and branching of the polymannose region of the glycopeptide occur apparently by a direct transfer of mannosyl units from GDP-mannose (155). The order of the formation of individual types of glycosidic bonds between the mannosyl units and the sizes of the side chains are most probably determined by the presence of individual mannosyl transferases and their substrate specificities.

The latter assumption comes from the results of experiments with yeast mannan-synthesizing enzyme systems where mannose and short mannosyl oligosaccharides with defined structures were used as exogenous acceptors for transfer of mannosyl units from GDP-mannose (81, 151, 193, 243). For example, free mannose can serve as the acceptor for the  $\alpha$ -1,2-mannosyltransferase (81, 151, 193) while the  $\alpha$ -1,6-mannooligosaccharides serve as substrated for both the  $\alpha$ -1,6-mannosyltransferase (81, 193) and the  $\alpha$ -1,2-mannosyltransferase (193). The mannotetraose containing an  $\alpha$ -1,3-linked mannosyl unit at the nonreducing end accepts a new mannosyl unit at position C-6 of the reducing terminal mannose (193). A mannosylphosphate transferase isolated from *S. cerevisiae* catalyzes the transfer of mannosyl-1-phosphate from GDP-mannose to position C-6 of the penultimate mannosyl unit in reduced  $\alpha$ -1,2-linked mannotetraose (129). No evidence was obtained that a lipid-bound mannosylphosphate derivative was involved in the reaction.

The molecular mechanisms of mannoprotein synthesis in other yeast species and different fungal genera have not been studied to such an extent as with *S. cerevisiae*. In the biosynthesis of phosphomannan of *Hansenula* yeast species, GDP-mannose serves as the donor of both mannosyl units and the phosphate (41, 138, 179), the latter being transferred from the sugar nucleotide in the form of mannosyl-1-phosphate (41).

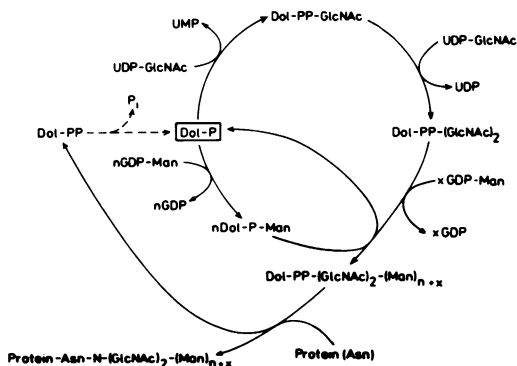


FIG. 7. Scheme showing the possible sequence in biosynthesis of the "inner core" of yeast mannan and the role of glycosylated dolichol phosphates. Dol-P, Dolichol phosphate; Dol-PP, dolichol pyrophosphate;  $P_i$ , inorganic phosphate; Man, mannose; Asn, asparagine.  $x + n = 0$  to 17.

The enzyme system isolated from *Hansenula* species catalyzes the formation of both *N*-glycosidically and *O*-glycosidically linked carbohydrate moieties (40) with the participation of dolichol monophosphate as the lipid intermediate (42, 43).

A particulate enzyme system isolated from *Cryptococcus laurentii* catalyzes the transfer of mannose from GDP-mannose to endogenous as well as to exogenous low-molecular-weight acceptors with the formation of  $\alpha$ -1,2-,  $\alpha$ -1,3-, and  $\alpha$ -1,6-mannosyl linkages and a mannosyl-xylosyl linkage (225, 226). In the same system UDP-xylose and UDP-galactose serve as the donors of xylosyl (227) and galactosyl (211) units for the formation of cell wall heteropolysaccharides. In *Kluyveromyces lactis* the UDP-GlcNAc serves as the source of terminal GlcNAc units in the side chains of the wall mannan (243).

Hyphal fungi contain relatively little glycoprotein in their cell walls when compared with yeasts (15). Owing to this fact, studies dealing with the mechanism of biosynthesis of the glycoprotein components of hyphal walls are comparatively scarce. In *Aspergillus niger*, GDP-mannose has been reported (160) to serve as the donor in the transfer of mannosyl units to endogenous acceptors. Formation of a polyprenol phosphate-mannose serving as the intermediate in some mannosyltransferase reactions has also been observed (158, 159). A similar mannosyl-1-phosphorylpolyisoprenol has been discovered also in *N. crassa*, where it apparently serves as an obligatory intermediate in the transfer of mannosyl units to peptide acceptors (99).

A particulate enzyme preparation isolated from *Penicillium charlesii* catalyzed the incorporation of mannosyl units from GDP-mannose into both endogenous acceptors and added peptidophosphogalactomannan (92). Under these conditions, the transfer of mannose into both the *O*-glycosidically linked oligosaccharide and the phosphogalactomannan region of the glycopeptide acceptor was observed. About 10% of the mannosyltransferase activity could be solubilized from the membranes by treatment with Triton X-100. The solubilized enzyme incorporated mannosyl units from GDP-mannose predominantly into the phosphogalactomannan region of the acceptor, whereas the remainder of the activity, not solubilized by the detergent, catalyzed the formation of mannopyranosyl-(seryl/threonyl) linkages in the peptidophosphogalactomannan (93). So far, no participation of mannosyl-linked lipid intermediates in these reactions has been detected.

(ii) **Regulation.** The biosynthesis of yeast mannan in vivo appears to depend intimately on

undisturbed protein synthesis. When the formation of proteins is blocked by cycloheximide in intact cells (74) or protoplasts (80, 162, 256, 261), the formation and extracellular appearance of mannoproteins and mannoprotein enzymes are halted after a short delay. Cycloheximide does not affect the activity of the isolated mannan-synthesizing enzyme system (80, 232); neither does it influence the relatively low turnover of the corresponding enzymes (75). Hence, it is probable that blocking of mannan synthesis by cycloheximide reflects exhaustion of the pool of peptides serving as acceptors for mannosyl transfer. The inhibition of mannoprotein synthesis by cycloheximide is accompanied by a marked increase in the pools of GDP-mannose and UDP-GlcNAc (232, 256), the glycosyl donors for biosynthesis of carbohydrate portions of the mannan.

Mannosylation of at least some intracellular proteins seems to be a necessary prerequisite for their transport across the plasma membrane to the cell exterior. Impediment of mannosylation by 2-deoxyglucose in the presence of glucose or fructose as respective carbon sources (80, 139, 141, 162, 261) or by tunicamycin (142, 143) results in inhibition of the formation and secretion of the exocellular mannoprotein enzymes invertase and acid phosphatase.

Whereas tunicamycin interferes with the glycosylation of proteins (142, 143) through inhibition of the *N*-acetylglucosaminyl-transferase catalyzing the formation of the intermediate dolichol pyrophosphate-GlcNAc (153), the inhibition of glycosylation by 2-deoxyglucose seems to be of a more complex nature (139). 2-Deoxyglucose inhibits the mannosylation of proteins by interfering with the conversion of glucose to mannose at the level of their 6-phosphates (141, 273). In addition, the GDP-2-deoxyglucose formed in cells cultivated in the presence of 2-deoxyglucose (31) could act as a competitive inhibitor in the mannosylation reaction (35, 150, 217).

There seems to operate quite an effective feedback mechanism controlling the synthesis of non-glycosylated forms of extracellular enzymes under conditions of inhibited glycosylation, since no substantial accumulation of carbohydrate-free forms of these enzymes in the cytoplasm takes place (142, 162, 261). The existence of such a regulatory mechanism is strongly indicated also by the finding that a mannose-deficient mutant of *Schizosaccharomyces pombe* is unable to synthesize the carbohydrate-free form of acid phosphatase, despite derepression conditions, when mannosylation is blocked by the absence of mannose in the growth medium (224).

Somewhat contradictory results were obtained by Moreno et al. (189), who observed an accumulation of the light and intermediate, partially glycosylated forms of yeast invertase at the expense of heavy, fully glycosylated enzyme when cells were incubated in the presence of 2-deoxyglucose.

(iii) **Localization of reactions.** The experimental evidence indicates that the biosynthesis of mannan starts at an early stage in the formation of its protein moiety. Short pulses of [ $^{14}\text{C}$ ]mannose to growing yeast protoplasts followed by their lysis and subcellular fractionation revealed that the nascent polypeptide is glycosylated by small amounts of mannose, glucosamine, and, surprisingly, glucose (217). This initial glycosylation apparently represents the formation of linkage regions between the protein and carbohydrate moieties in the mannan, as judged from the participation of dolichol phosphate-mannose in the glycosylation reaction (147).

The cellular location of mannosyltransferases was studied by using different techniques, including autoradiography (136), cytochemical staining (267), and subcellular fractionation on urografin gradients (61, 136, 148). The latter experiments have shown that the highest specific activity of mannosyltransferases resides in the membranes of the endoplasmic reticulum; however, a significant portion of activity has been found also in other membrane fractions, including the plasmalemma.

Analysis of products synthesized by different membrane fractions confirmed the observation (147) that the membranes of the endoplasmic reticulum possess the highest specific activity of those mannosyltransferases requiring dolichol phosphates as intermediates for mannosyl transfer (148). On the other hand, the cytoplasmic vesicles and plasmalemma exhibited increased activity of enzymes participating in the elongation of oligo- and polymannose chains, not requiring the participation of dolichol phosphates (148, 174).

These data imply that the formation of manoproteins is a vectorial process involving the transfer of nascent polypeptides along the membranes of the rough and smooth endoplasmic reticula and the Golgi cisternae and finally transport across the plasmalemma, accompanied on this route by stepwise addition of mannosyl units to the growing carbohydrate chains.

The role of glycosylation in this translocation is far from clear. The results obtained with yeasts as models seem to support Eylar's (78) proposal that the glycosylation of proteins enables their passage to the cell exterior. Neverthe-

less, the mechanism by which control over glycoprotein secretion by glycosylation is executed remains still a subject of speculation. One of the possibilities is that the glycosylation of nascent, membrane-associated proteins facilitates, by mutual interaction of hydrophilic carbohydrate groups, the pinching-off and vesiculation of portions of the membranes of the endoplasmic reticulum whereby the glycosylated parts of the glycoproteins are facing the vesicle interior and the protein moieties remain temporarily associated with the enclosing membrane.

Another open question is the mechanism of glycoprotein secretion. Two models for glycoprotein transport have been proposed: (i) reverse pinocytosis of carrier vesicles which after fusion with the plasmalemma would discharge their contents into the cell exterior (see, for example, Grove et al. [104]), which would be a process analogous to glycoprotein secretion in higher organisms (175), (ii) free "diffusion" of glycoprotein macromolecules across the plasmalemma without involvement of secretory vesicles (184).

The participation of vesicles in glycoprotein transport in fungi seems to be well established. Numerous examples can be found in the literature showing an accumulation of vesicles near the extending zones of the cell walls (28, 57, 114, 121, 176, 187). Chemical and histochemical analyses of the contents of the secretory vesicles shows that they contain a large proportion of cell wall matrix material (28, 62, 104). Another function of vesicles could be that they might supply new material for the extension of the plasmalemma (17, 253).

What forces direct the secretory vesicles towards the sites of active cell wall growth remains to be established. Theoretically, the movement of organelles in the cytoplasm could be organized either by cytoplasmic streaming, by a system of microtubules, or by electric forces. The existence of an electrical potential between the apex and subapical regions in fungal hyphae (241, 253) would speak in the favor of the latter possibility. However, the participation of microtubules in directing the secretory vesicles cannot be excluded, especially in view of the finding of Byers and Goetsch (45), who observed bundles of microtubules extending from the nuclear plaque into early buds in *S. cerevisiae*.

### Formation of Wall Fabric

With regard to the structural complexity and mechanical integrity of the cell wall, it is undoubted that the final steps in the formation of the cell wall take place extracellularly, i.e., in situ. The formation of the cell wall fabric from its macromolecular constituents must involve

association of subunits, their cross-linking, and their aggregation into a supramolecular structure with defined chemical composition and morphology.

In contrast to the well-established presence of polysaccharidehydrolases in the cell walls and periplasmic space (e.g., 14, 83, 85, 168, 206), there is, so far, no evidence about the existence of ligase-type enzymes catalyzing the formation of chemical linkages between the individual macromolecular components of the wall. Besides the noncatalytic formation of disulfide bridges between the protein moieties of wall glycoproteins, a great role undoubtedly is played by the self-assembly of subunits in the formation of wall fabric with participation of physicochemical interactions. As such can be considered, for example, the formation of fibrils of insoluble skeletal polysaccharides by rapid crystallization of formed subunits (71) or polysaccharide fragments produced by the attack of polysaccharide hydrolases in the process of cell wall extension.

Some information about the process of cell wall assembly in fungi could be obtained by experiments in vitro involving dissociation and reassociation of wall components. Similar experiments have been successful, so far, with relatively simple biological structures, such as ribosomes (144), virus particles (72), bacterial flagella, and the like (145). Hills (113) was able to demonstrate in vitro dissociation and reassembly of glycoprotein-composed cell walls of *Chlamydomonas reinhardi* after their dissolution in 8 M lithium chloride and subsequent dialysis of the solution against water. A "nucleating agent," insoluble in 8 M lithium chloride, was required to initiate cell wall reassembly. However elegant they may be, such experiments do not necessarily indicate the real sequence of events in the assembly process when proceeding in vivo.

Although at present no means exist to dissociate the complex fungal walls without denaturation of their basic components, certain possibilities for overcoming this obstacle are represented by the use of fungal protoplasts. The protoplasts under suitable conditions produce and secrete practically all the cell wall constituents (146, 198, 265). The insoluble wall components remain during this process in the form of a microfibrillar network in close proximity to the protoplast surface while the soluble components of glycoprotein nature diffuse away into the surrounding medium. When the fibrillar polysaccharide net formed at the protoplast surface is dense enough or when the diffusion of soluble material is prevented by an artificial permeability barrier, such as embedding the protoplasts in a gel, regeneration of the cell wall takes place

(198). The increased concentration of cell wall building elements at the surface of the protoplasts apparently facilitates their mutual contact and cross-linking by chemical and physicochemical interactions and leads to the formation of the new compact wall (for more comprehensive descriptions of the process of cell wall regeneration, see, for example, 198, 205, 263, 265).

The formation of new portions of the cell wall in vegetative fungal cells may involve a similar mechanism. The difference is that here the nascent cell wall constituents are being locally incorporated into the preexisting cell wall structure playing the role of template by ordering the proper position and assembly of the newly arrived macromolecules.

The number of chemical and physicochemical links in the cell walls apparently increases with the cell age, as is indicated by the observation that the older portions of the walls are more resistant to attacks by endogenous (207) as well as exogenous (44, 198, 263) polysaccharide hydrolases.

### Metabolic Stability of the Wall

Some bacteria exhibit extensive turnover of cell wall components during the active growth (e.g. 36, 274). Although the exact mechanism is not known, it is assumed that the autolytic enzymes (autolysins) present in the periplasmic space or in the wall cause the dissolution of wall material, its release into the medium, and its eventual reutilization by the cells.

The isolated cell walls of many fungi exhibit, due to the presence of different polysaccharide hydrolases, appreciable autolytic activity (e.g. 14, 83, 85, 206). Although these enzymes are supposed to be involved primarily in cell wall weakening during growth and other morphogenetic processes, a question arises as to whether their action on the cell wall could cause appreciable turnover of the individual cell wall components and their exchange with the monomers in the cytoplasm during vegetative growth.

Pulse-chase experiments with cells of *Aspergillus clavatus* labeled with radioactive glucose revealed that glucose and GlcNAc became metabolically inert once they had been incorporated into the wall polymers (60). A similar conclusion has been drawn from experiments with cells of *S. cerevisiae* whose cell wall mannan was selectively labeled with 2-deoxy-D-[<sup>3</sup>H]glucose, D-[<sup>14</sup>C]mannose, or when the cell wall was universally labeled with D-[<sup>14</sup>C]glucose (140). After transferring the yeast cells from radioactive into non-radioactive growth media, the radioactivity in the cell walls persisted for at least three generations.

Nevertheless, there are indications that under

limiting conditions, or in certain periods of the life cycle, the cell wall polysaccharides may serve as a metabolic reserve. In a quantitative study on the changes of  $\alpha$ -1,3-glucan in the cell wall of *Aspergillus nidulans*, Zonneveld (278) has observed a marked decrease of  $\alpha$ -1,3-glucan content in the cell walls, accompanied by an increase of  $\alpha$ -1,3-glucanase activity during cleistothecium development induced by glucose depletion from the medium.

Stationary-phase yeast cells when transferred to a fresh growth medium exhibit a sudden fall in the mannan content of their walls accompanied by an increase in  $\alpha$ -mannanase activity (257). A similar reutilization of the mannan fraction of yeast cell walls is observed in the course of starvation of the cells (167). The contents of glucose, xylose, and other saccharides in the cell wall gradually decrease also during prolonged spontaneous autolysis of the mycelium of *Aspergillus terreus* (145a). Capsular polysaccharides produced and released into the medium by some yeasts exhibit in some cases striking structural similarities with the cell wall components (205). Capsular material could either originate from the upper glycoprotein layer of the walls or represent surplus production of cell wall components.

These examples point out that the cell wall of fungi is by no means a stable, metabolically inert structure serving merely as a protective shell for the metabolically active protoplast. It is a "living" organelle whose functions may vary with environmental conditions and in the course of cell and life cycles.

## MORPHOGENETIC ASPECTS

### Topology of Wall Growth

From the accumulated knowledge on the process of cell wall construction, it becomes increasingly obvious that the wall's morphology depends not only on its chemical structure but also largely on the mode of its construction, i.e., on the topology of wall growth.

The idea that fungal hyphae grow by incorporating new cell wall material at the wall apex has become one of the basic dogmas of fungal cytology (17, 212, 242, 253). The data supporting this view originate from numerous studies based on microscopy (97, 212), autoradiography (19, 22, 89, 90, 100, 131), and fluorescent labeling (90, 121, 173, 253), among other methods. On the other hand, a spherical shape is considered to be the result of uniform layering of new cell wall material over the entire cell surface (19, 22). Autoradiographic observations indicate that the ellipsoidal shape of yeast cells is acquired by participation of both polarized growth and

spherical extension (33, 79, 251).

Supposing that in principle the molecular mechanisms involved in the process of cell wall formation are essentially the same or very similar in various morphological types, a basic question arises about how these processes are regulated so that the characteristic shape is generated.

Several hypotheses have been put forward in the past to explain the mechanism of cell wall formation and morphogenesis in fungi. Johnson (125) suggested that the cell wall is elaborated with participation of both synthetic and lytic processes. According to his hypothesis, the preexisting rigid cell wall structure is first attacked by specific cell wall-bound polysaccharide hydrolases, and the gaps formed are then filled with newly synthesized cell wall material. The continual repetition of this process would ensure the growth of the cell wall without disturbing its overall integrity.

The results from different laboratories have shown that, indeed there exists a delicate balance between the synthetic and lytic processes in the cell wall (21). Whenever this balance is disturbed, morphological aberrations or lysis or both occur. For example, inhibition of chitin synthesis by polyoxin D causes bulging and lysis of hyphal cells of *M. rouxii* (20). Similarly, inhibition of wall glucan synthesis in yeast by 2-deoxyglucose or 2-deoxy-2-fluoroglucose leads to morphological changes and often to lysis of cells (32, 34, 35, 125, 239). Inhibition of chitin synthesis and stimulation of cell wall hydrolases by increased temperature causes distortions and lysis of cell walls in hyphae of *A. nidulans* (132). On the other hand, inhibition of wall-plasticizing glucanases by  $\delta$ -gluconolactone causes cessation of wall growth in auxin-induced yeast cells (237). The morphogenic effects of polyoxin D and the deoxyanalogs of glucose in fungal cells resemble the action of penicillin on the cell wall formation and morphogenesis in bacteria (e.g., 228).

To explain the morphological development of fungal cell walls, Bartnicki-Garcia (17) postulated the so-called unitary model of wall growth. In his model cell wall growth is considered as a result of cumulative action of minute hypothetical units of wall growth at the cell surface. The units of wall growth are supposed to contain both lytic and synthetic enzymes involved in the formation of wall fabric. Accordingly, cell morphology is determined by the distribution pattern of the hypothetical growth units. Thus, apical growth would be the result of concentration of growth units at the hyphal tip, whereas spherical morphology would result from uniform distribution of growth units over the whole cell surface. Although plausible, the above hypoth-

esis lacks an explanation of how the distribution pattern of postulated growth units is determined and maintained and how their activity is regulated.

From the observation that the yeast form of *Paracoccidioides brasiliensis* contains  $\alpha$ -1,3-glucan as the main cell wall component, whereas the mycelial wall of the same species is composed predominantly of  $\beta$ -1,3-glucan (56), Kanetsuna et al. (128) proposed a hypothesis according to which the temperature-induced yeast-to-mycelial reversion is due to local activation of  $\beta$ -glucan synthesis at the expense of  $\alpha$ -glucan formation.

If we are to find a more realistic answer to the problem of regulation of morphological development, we must consider the characteristics and regulatory properties of the enzymes involved in cell wall construction, as well as all factors that could influence this process in living cells. A good starting point in this direction might be the already described hypothesis of Cabib and co-workers (46, 50, 51) explaining the formation of yeast primary septum by temporally and spatially localized activation of plasmalemma-bound chitin synthase. Although originally intended to explain only the special case of septum formation, the concept of regulation of morphogenetic events through changes in the activities of preexisting polysaccharide synthases could find a more general application. The latter possibility seems to be supported by numerous, often unaimed observations made with fungi and fungal protoplasts, as can be seen from the following examples.

Removal of the fungal cell wall by enzymic or other suitable treatment liberates protoplasts that under certain conditions are capable to regenerate their cell walls. The polysaccharide synthases are set into operation, and usually after a short period of time a network of polysaccharide microfibrils is formed on the protoplast surface (e.g., 198, 265). In all cases, however, the formation of new a cell wall around the protoplast is isodiametric, i.e., the shape of the regenerated cell is independent from the morphology of the parent cell type (e.g. 88, 94, 198, 265); A. Svoboda and O. Nčas, Proc. Int. Symp. Yeasts 4th, Vienna, Austria, 1974, D13, p. 215). The shape of the regenerated protoplast is rounded or irregular, depending on the properties of the surrounding medium, and the thickness of the newly formed wall is uniform over the whole protoplast surface. It is usually only after several generations that regenerated cells regain their original characteristic morphology (Fig. 8).

Experimental evidence shows that the for-

mation of skeletal, microfibrillar components of the wall on the protoplast surface is to a great degree independent from protein synthesis (64, 198, 199). In some cases it was observed that blocking protein synthesis in regenerating protoplasts (64, 256) or intact cells (74) even stimulates to some extent the formation of skeletal wall polysaccharides. The diminished presence of glycoprotein wall components at the surface of the protoplast of *Candida utilis* regenerating in liquid medium is accompanied by an about 20 times higher accumulation of chitin in the regenerated walls in comparison with the chitin content of normal walls (95). An increased formation of chitin wall component was observed also in protoplasts of *S. cerevisiae* regenerating in a liquid medium containing [ $^{14}$ C]glucose as the sole carbon source (V. Farkaš and A. Svoboda, unpublished data).

Another example of the independence of skeletal wall polysaccharide formation from protein synthesis is provided by the encystment of zoospores of *Blastocladiella emersonii*, *Phytophthora palmivora*, and others. In these cases also, blocking of protein synthesis by cycloheximide does not prevent the formation of a chitinous sheath during the encystment of zoospores (166, 230, 244).

A conclusion may be drawn from these observations that the enzymes responsible for skeletal wall polysaccharide synthesis are synthesized constitutively and that they are relatively stable against turnover. The absence of protein synthesis does not inhibit their function; on the contrary, in some cases the absence of protein synthesis even stimulates their function. At this point it is tempting to speculate that this property of fungal cell wall-synthesizing enzymes may be of vital importance for survival in many fungi; it enables them to complete the life cycle and undergo sporulation even under unfavorable conditions, such as the limitation of cell metabolism by lack of nutrients or other negative circumstances.

Furthermore, the above results seem to indicate that it is essentially the presence of the cell wall or contact with some extracytoplasmic components that hinders the expression of cryptic polysaccharide synthases in the plasmalemma.

Conceivably, loosening the contact between the cell wall and plasmalemma could bring about the activation of polysaccharide synthesis. Distortion of the contact between the plasmalemma and cell wall can be achieved not only by complete removal of the wall, as is the case with protoplasts, but also in the intact cells by purely physical means, such as osmotic shock or mechanical vibration. For example, rapid transfer

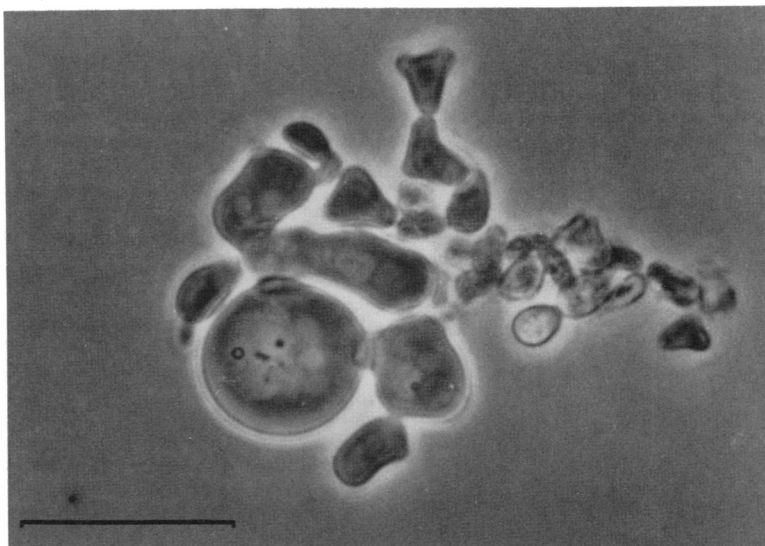


FIG. 8. *Regeneration and reversion of protoplasts of the yeast Trigonopsis variabilis after 40 h of incubation in osmotically stabilized growth medium containing 30% gelatin. (Courtesy of A. Svoboda.) Bar represents 10  $\mu$ m.*

of cells from diluted buffer into medium with high osmolarity causes plasmolysis in *A. niger* whereby the normal apical pattern of wall growth is disturbed and formation of abnormal numbers of branchings and septa is induced (131, 246). Repeated changes in the osmolarity of the growth medium induces the formation of multiple layers of wall material on the surface of regenerating yeast protoplasts (A. Svoboda, unpublished data).

Autoradiography of hyphal cell walls isolated by mechanical disintegration of *M. rouxii* cells incubated with UDP-[ $^3$ H]GlcNAc revealed that in a great number of cases the apical pattern of labeling was lost, possibly as a consequence of the mechanical treatment of cells before autoradiography (unpublished data of J. McMurrugh, A. Flores-Carreón, and S. Bartnicki-Garcia, mentioned in reference 17).

To a special category belong the various morphogenetic effects induced in fungi by certain antibiotics. Such changes as curling, bulging, abnormal spherical growth, frequent branching, and increase in wall thickness have been observed when different antibiotics have been applied to growing fungi (i.e. 11–13, 20, 26, 29, 130, 131, 252). In most cases the mechanisms of action of different morphogenetic antibiotics on the processes of cell wall formation in fungi have not been studied in detail, if at all. Nevertheless, from comparison of the data it becomes clear that inhibition of protein (27, 73, 74, 131, 246, 256) or glycoprotein (130, 142) synthesis usually

has as a consequence increased synthesis of skeletal wall polysaccharides, very often accompanied by a change in wall morphology.

In these cases it is possible to obtain delocalized synthesis of structural wall polysaccharides, apparently without disturbing the contact between the cell wall and the plasmalemma. Here it can be hypothesized that direct or indirect inhibition of protein synthesis may lead to depletion of some metabolically unstable proteinaceous extracytoplasmic components inhibiting the activity of cryptic polysaccharide synthases in the plasmalemma. A similar explanation may be offered for the delocalized synthesis of chitin observed at nonpermissive temperature in a temperature-sensitive mutant of *S. cerevisiae* (240) as well as for the observation that yeast cells in which deoxyribonucleic acid and protein syntheses were inhibited by inositol deficiency continued the synthesis of wall glucan, resulting in the formation of abnormally thick cell walls (66).

#### Speculations About the Possible Regulatory Mechanism

If we state that the cell wall determines the shape of the cell, we must bear in mind that the reverse is even more true: the cell determines the shape of the wall. The mechanism by which this control of the cell over the cellular morphology is executed represents one of the most intriguing problems of modern biology.

The experimental data summarized in the preceding pages strongly indicate that the key role in the regulation of fungal morphogenesis is played by the activation or inactivation or both of polysaccharide synthases located at the plasmalemma. Whereas the activation proceeds in the intact cells apparently through enzymatic disturbance of the contact between the plasmalemma and the constituents of the cell wall or periplasm, the inactivation must involve the reverse process, i.e., the combination of plasmalemma-bound polysaccharide synthases with specific proteinaceous inhibitors present in the periplasm or the cell wall. The proteinaceous nature of these postulated inhibitors is judged mainly from the fact that blocking protein (or glycoprotein) synthesis leads to activation of cryptic polysaccharide synthases and delocalized synthesis of wall. Although there is, so far, no experimental evidence for the existence of such inhibitors, their presence is clearly missing in the present schemes of regulation of cell wall polysaccharide synthesis (viz., Fig. 4). An inhibitory protein acting directly on active chitin synthase was isolated from the cytoplasm of mycelial (182) as well as yeast (164) cells of *M. rouxii*. How the activation-inactivation processes could actually participate in the regulation of cell wall growth is the subject of the following speculations.

The morphological development of the fungal cell begins by the activation of cell wall synthesis at some site on the preexisting cell wall. It can be assumed that the outgrowth of the new cell wall is preceded by the concentrated action of cell wall polysaccharide hydrolases at the given site. The localized dissolution of the wall polymers causes a loosening of the contact between the cell wall and plasmalemma and, in turn, activates the cryptic polysaccharide synthases located in plasmalemma at the given site. This sequence of events would create on the cell surface a region of active wall growth, henceforth called the "primary growth region" (Fig. 9). As a result of this activity, a tiny bud is formed on the surface of the preexisting wall.

Cytoplasmic vesicles containing enzymes, inhibitors, plasmalemma precursors, and wall matrix material move to the primary growth region, fuse with the plasmalemma, and discharge their contents into the periplasmic space. The polysaccharide synthases might be incorporated into plasmalemma in the fully active state, or they may be stabilized and become active only in association with lipophilic components of the plasmalemma. Such a mode of activation is common with some plasmalemma-bound enzymes (70, 137).

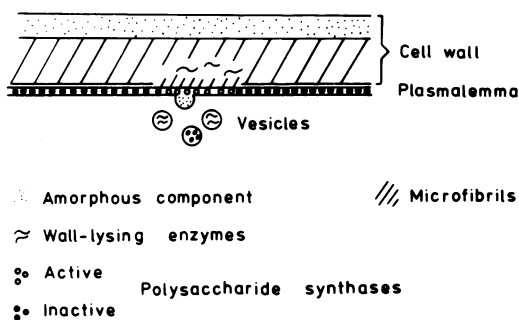


FIG. 9. Diagram illustrating formation of a primary growth region in a preexisting cell wall.

The highest frequency of fusion of carrier vesicles with the plasmalemma would be at the bud apex (e.g., 17, 253). If the polysaccharide synthases coming to the newly formed apex get into contact with the postulated extracytoplasmic inhibitors and become rapidly inactivated, a gradient of polysaccharide synthase activity is formed on the surface of the bud. The highest synthesizing activity would be at the apex, where new active molecules of polysaccharide synthases are supplied. Consequently, the hemispherical bud would turn into a hypha (Fig. 10A).

If, on the other hand, the concentration of inhibitors or the rate of inactivation of polysaccharide synthases at the apex is low, all polysaccharide synthases incorporated into plasmalemma remain active, resulting in spherical growth of the bud (Fig. 10B). It can be imagined that any intermediate rates of inactivation of nascent polysaccharide synthases would result in the formation of an ellipsoid.

It is highly probable that the distribution pattern of active polysaccharide synthases at the plasmalemma is reflected also in the rate of cell wall growth. The latter term is meant here as the number of precursor molecules incorporated into the whole cell wall in a unit of time. In hyphal walls, where rapid inactivation of polysaccharide synthases causes restriction of the growth zone to the wall apex, the constant rate of cell wall growth is maintained practically over the whole growth period (253).

On the other hand, in spherical or nearly spherical cells, where the number of active polysaccharide synthases in the plasmalemma constantly increases, the rate of cell wall growth can be expected to increase proportionally to the increase in plasmalemma area for most of the cell cycle. Data supporting this assumption can be deduced from results obtained with synchronously growing yeasts (30, 110, 112, 238).

The regulatory mechanism suggested here

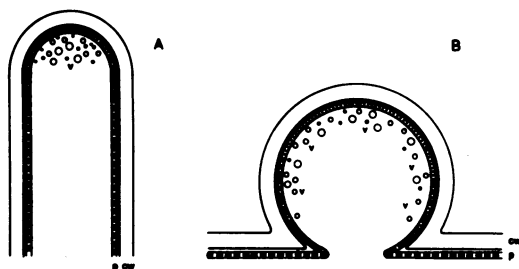


FIG. 10. Hypothetical distribution of active (○) and inactive (●) molecules of polysaccharide synthases in the plasmalemma of hyphae (A) and spherical cells (B). v, Cytoplasmic vesicles carrying enzymes and building material to the plasmalemma; p, plasmalemma; cw, cell wall.

represents an attempt to explain morphological development in fungi in terms of molecular interactions. It is limited in many respects and lacks direct experimental support. The restriction of the problem of fungal morphogenesis to the level of the cell wall necessarily brings many simplifications into our speculations and the experimental approach. It should be kept in mind always that formation of the cell wall is not an isolated process but is the result of metabolic activity of the whole cell.

### SUMMARY AND CONCLUSIONS

The fungal cell wall is a complex structure composed mainly of polysaccharides and their chemical complexes with proteins. The crystalline polysaccharides, chitin and  $\beta$ -glucans, constitute the skeletal portion of the wall, whereas the amorphous polysaccharides and protein-polysaccharides are components of the wall matrix.

The biosynthesis of each of these components proceeds in a different way. The protein-polysaccharide complexes are polymerized from their activated precursors by enzymes located in the rough and smooth endoplasmic reticula. The polymerized products are packed into vesicles derived from membranes of the endoplasmic reticulum and conveyed to sites on the plasmalemma adjacent to the growing regions of the wall.

Biosynthesis of skeletal polysaccharides is catalyzed by constitutively formed polysaccharide synthases uniformly distributed in the plasmalemma. The unique property of these enzymes is that they can exist in an active or in temporarily inactive, zymogen, state. Only the portion of polysaccharide synthases located at the growth zone is active during wall growth. In hyphal cells the growth of the wall is restricted to the apical region of the wall, whereas in

spherical cells new cell wall material is being incorporated over the whole surface of the wall. The activation-inactivation process with polysaccharide synthases seems reversible, and it is assumed that it represents the principal mechanism by which the fungal morphogenesis is regulated.

In this review I have tried to summarize briefly our present knowledge on the extremely complicated process of cell wall formation in fungi. In spite of the great progress that has been made in recent years in understanding the mechanism of cell wall formation in fungi, numerous detailed questions have to be answered before we can compose a more precise picture of the whole process. Future research will undoubtedly bring, among other solutions, the answers to such questions as: (i) the mechanisms of biosynthesis of various cell wall polymers and the participation of glycosylated phospholipids as intermediates in individual transglycosylation reactions, (ii) the participation of cellular organelles in the biosynthesis of individual cell wall polymers, (iii) the mechanism of transport of prefabricated wall polymers from the site of formation to the cell exterior, (iv) the polymerization of subunits in the wall and formation of wall fabric, and (v) the regulation of the biosyntheses of individual cell wall components with relation to morphological development and cell and life cycles.

To achieve these goals would necessarily require the exploitation of all the existing methodological potential of the modern biological sciences. Not only is the effort expended in this research likely to be rewarding in this special field of microbiology, but the knowledge obtained can contribute to a better understanding of related processes in types of cells other than fungi.

### ACKNOWLEDGMENTS

I thank A. Svoboda for many useful discussions, W. Tanner for the communication of previously unpublished results, S. Bartnicki-Garcia and A. Svoboda for original photographic material, and A. P. J. Trinci for permission to reproduce one figure from his paper. A part of this work was written during my stay at the Department of Microbiology, Attila József University, Szeged (Hungary), and I thank L. Ferenczy, head of that Department, for providing ideal conditions for my work and giving me access to his private collection of reprints and scientific literature. Last but not least, I thank my wife for her help and encouragement throughout the work on this review.

### LITERATURE CITED

1. Algranati, I. D., H. Carminatti, and E. Cabib. 1963. The enzymic synthesis of yeast mannan.

- Biochem. Biophys. Res. Commun. 12:504-509.
2. Anné, J., H. Eyssen, and P. De Somer. 1974. Formation and regeneration of *Penicillium chrysogenum* protoplasts. Arch. Microbiol. 98: 159-166.
  3. Archer, D. B. 1977. Chitin biosynthesis in protoplasts and subcellular fractions of *Aspergillus fumigatus*. Biochem. J. 164:653-658.
  4. Arnold, W. N. 1972. The structure of the yeast cell wall. Solubilization of a marker enzyme  $\beta$ -fructofuranosidase, by the autolytic enzyme system. J. Biol. Chem. 247:1161-1169.
  5. Aronson, J. M. 1965. The cell wall, p. 49-76. In G. C. Ainsworth and A. D. Sussman (ed.), The fungi, vol. 1. Academic Press Inc., New York and London.
  6. Babczinski, P., and W. Tanner. 1973. Involvement of dolichol monophosphate in the formation of specific mannosyl linkages in yeast glycoproteins. Biochem. Biophys. Res. Commun. 54:1119-1124.
  7. Bacon, J. S. D., V. C. Farmer, D. Jones, and I. F. Taylor. 1969. The glucan components of the cell wall of baker's yeast (*Saccharomyces cerevisiae*) considered in relation to its ultrastructure. Biochem. J. 114:557-567.
  8. Bálint, Š., V. Farkaš, and Š. Bauer. 1976. Biosynthesis of  $\beta$ -glucans catalyzed by a particulate enzyme preparation from yeast. FEBS Lett. 64:44-47.
  9. Ballou, C. E. 1976. Structure and biosynthesis of the mannan component of the yeast cell envelope. Adv. Microb. Physiol. 14:93-158.
  10. Ballou, C. E., and W. C. Raschke. 1974. Polymorphism of the somatic antigen of yeast. Science 184:127-134.
  11. Baráthová, H., and V. Betina. 1976. Antibiotic-induced changes of mycelial growth of *Botrytis cinerea*. Folia Microbiol. (Prague) 21:355-361.
  12. Baráthová, H., V. Betina, Z. Baráth, and P. Nemec. 1975. Morphogenic effects of ramihyphal A in filamentous fungi. Folia Microbiol. (Prague) 20:97-102.
  13. Baráthová, H., V. Betina, and P. Nemec. 1969. Morphological changes induced in fungi by antibiotics. Folia Microbiol. (Prague) 14:475-483.
  14. Barras, D. R. 1972. A  $\beta$ -glucan endohydrolase from *Schizosaccharomyces pombe* and its role in cell wall growth. Antonie van Leeuwenhoek J. Microbiol. Serol. 38:65-80.
  15. Bartnicki-Garcia, S. 1968. Cell wall chemistry, morphogenesis and taxonomy of fungi. Annu. Rev. Microbiol. 22:87-108.
  16. Bartnicki-Garcia, S. 1970. Cell wall composition and other biochemical markers in fungal phylogeny, p. 81-103. In J. B. Hasborne (ed.), Phytochemical phylogeny. Academic Press Inc., New York and London.
  17. Bartnicki-Garcia, S. 1973. Fundamental aspects of hyphal morphogenesis. Symp. Soc. Gen. Microbiol. 23:245-267.
  18. Bartnicki-Garcia, S., C. E. Bracker, E. Reyes, and J. Ruiz-Herrera. 1978. Isolation of chitosomes from taxonomically diverse fungi and synthesis of chitin microfibrils in vitro. Exp. Mycol. 2:173-192.
  19. Bartnicki-Garcia, S., and E. Lippman. 1969. Fungal morphogenesis: cell wall construction in *Mucor rouxii*. Science 165:302-304.
  20. Bartnicki-Garcia, S., and E. Lippman. 1972. Inhibition of *Mucor rouxii* by polyoxin D: effects on chitin synthetase and morphological development. J. Gen. Microbiol. 71:301-309.
  21. Bartnicki-Garcia, S., and E. Lippman. 1972. The bursting tendency of hyphal tips of fungi: presumptive evidence for a delicate balance between wall synthesis and wall lysis in apical growth. J. Gen. Microbiol. 73:487-500.
  22. Bartnicki-Garcia, S., and E. Lippman. 1977. Polarization of cell wall synthesis during spore germination of *Mucor rouxii*. Exp. Mycol. 1: 230-240.
  23. Bartnicki-Garcia, S., and I. McMurrough. 1971. Biochemistry of morphogenesis in yeast, p. 441-491. In A. H. Rose and J. S. Harrison (ed.), The yeasts, vol. 2. Physiology and biochemistry of yeasts. Academic Press Inc., New York and London.
  24. Behrens, N. H., and E. Cabib. 1968. The biosynthesis of mannan in *Saccharomyces cerevisiae*. J. Biol. Chem. 243:502-509.
  25. Behrens, N. H., H. Carminatti, R. J. Stanelloni, L. F. Leloir, and A. I. Cantarella. 1973. Formation of lipid-bound oligosaccharides containing mannose. Their role in glycoprotein synthesis. Proc. Natl. Acad. Sci. U.S.A. 70: 3390-3394.
  26. Bent, K. J., and R. H. Moore. 1966. The mode of action of griseofulvin. Symp. Soc. Gen. Microbiol. 16:82-110.
  27. Berthe, M. C., N. Ansary, and R. Bonaly. 1977. Effect of cycloheximide on the chemical composition of the cell walls of two strains of *Rhodotorula*. Mycopathologia 62:167-172.
  28. Beteta, P., and S. Gascón. 1971. Localization of invertase in yeast vacuoles. FEBS Lett. 13: 297-300.
  29. Betina, V., and D. Mičková. 1973. Morphogenetic activity of cytochalasins, cyanein and monorden in *Botrytis cinerea*. Z. Allg. Mikrobiol. 13:287-298.
  30. Biely, P. 1978. Changes in the rate of synthesis of wall polysaccharides during the cell cycle of yeast. Arch. Microbiol. 119:213-214.
  31. Biely, P., and Š. Bauer. 1968. The formation of guanosine diphosphate 2-deoxy-D-glucose in yeast. Biochim. Biophys. Acta 156:432-434.
  32. Biely, P., V. Farkaš, and Z. Krátky. 1974. Some aspects of wall formation in *Schizosaccharomyces pombe*. A study with 2-deoxyglucose. Biologia (Bratislava) 29:919-925.
  33. Biely, P., J. Kovařík, and Š. Bauer. 1973. Cell wall formation in yeast. An electron microscopic autoradiographic study. Arch. Mikrobiol. 94:365-371.
  34. Biely, P., J. Kovařík, and Š. Bauer. 1973. Lysis of *Saccharomyces cerevisiae* with 2-deoxy-2-fluoro-D-glucose, an inhibitor of the cell wall

- glucan synthesis. *J. Bacteriol.* **115**:1108-1120.
35. Biely, P., Z. Krátky, J. Kovarik, and Š. Bauer. 1971. Effect of 2-deoxyglucose on cell wall formation in *Saccharomyces cerevisiae* and its relation to cell growth inhibition. *J. Bacteriol.* **107**:121-129.
  36. Boothbay, D., L. Daneo-Moore, M. L. Higgins, J. Coyette, and G. D. Shockman. 1973. Turnover of bacterial peptidoglycans. *J. Biol. Chem.* **248**:2161-2169.
  37. Bowden, J. K., and B. Hodgson. 1970. Evidence against the presence of fibres or chemically distinct layers in the cell wall of *Saccharomyces*. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **36**:81-108.
  38. Bracker, C. E., J. Ruiz-Hererra, and S. Bartnicki-Garcia. 1976. Structure and transformation of chitin synthetase particles (chitosomes) during microfibril synthesis in vitro. *Proc. Natl. Acad. Sci. U.S.A.* **73**:4570-4574.
  39. Braun, P. C., and R. A. Calderone. 1978. Chitin synthesis in *Candida albicans*: comparison of yeast and hyphal forms. *J. Bacteriol.* **133**:1472-1477.
  40. Bretthauer, R. K., and G. C. Tsay. 1974. Enzymatic transfer of mannose from guanosine diphosphate mannose to yeast mannan-protein complexes. *Arch. Biochem. Biophys.* **164**:118-126.
  41. Bretthauer, R. K., C. P. Kozak, and W. E. Irwin. 1969. Phosphate and mannose transfer from guanosine diphosphate mannose to yeast mannan acceptors. *Biochem. Biophys. Res. Commun.* **37**:820-827.
  42. Bretthauer, R. K., and S. Wu. 1975. Synthesis of the mannosyl-O-serine (threonine) linkage of glycoproteins from polyisoprenyl phosphate mannose in yeast (*Hansenula holstii*). *Arch. Biochem. Biophys.* **167**:151-160.
  43. Bretthauer, R. K., S. Wu, and W. E. Irwin. 1973. Enzymatic transfer of mannose from guanosine diphosphate mannose to dolichol phosphate in yeast (*Hansenula holstii*). A possible step in mannan biosynthesis. *Biochim. Biophys. Acta* **304**:736-747.
  44. Brown, J. P. 1971. Susceptibility of the cell walls of some yeasts to lysis by enzymes of *Helix pomatia*. *Can. J. Microbiol.* **17**:205-208.
  45. Byers, B., and L. Goetsch. 1975. Behavior of spindles and spindle plaques in the cell cycle and conjugation of *Saccharomyces cerevisiae*. *J. Bacteriol.* **124**:511-523.
  46. Cabib, E. 1975. Molecular aspects of yeast morphogenesis. *Annu. Rev. Microbiol.* **29**:191-214.
  47. Cabib, E. 1976. The yeast primary septum: a journey into three-dimensional biochemistry. *Trends Biochem. Sci.* **1**:275-277.
  48. Cabib, E., and B. Bowers. 1971. Chitin and yeast budding. Localization of chitin in yeast bud scars. *J. Biol. Chem.* **246**:152-159.
  49. Cabib, E., and B. Bowers. 1975. Timing and function of chitin synthesis in yeast. *J. Bacteriol.* **124**:1586-1593.
  50. Cabib, E., and V. Farkaš. 1971. The control of morphogenesis: an enzymatic mechanism for the initiation of septum formation in yeast. *Proc. Natl. Acad. Sci. U.S.A.* **68**:2052-2056.
  51. Cabib, E., V. Farkaš, R. E. Ulane, and B. Bowers. 1973. Yeast septum formation as a model system for morphogenesis, p. 105-116. In J. R. Villanueva, I. Garcia-Acha, S. Gascón, and F. Uruburu (ed.) *Yeast, mould and plant protoplasts*. Academic Press Inc., New York and London.
  52. Cabib, E., and F. A. Keller. 1971. Chitin and yeast budding. Allosteric inhibition of chitin synthetase by a heat-stable protein from yeast. *J. Biol. Chem.* **246**:167-173.
  53. Cabib, E., R. Ulane, and B. Bowers. 1973. Yeast chitin synthetase. Separation of the zymogen from its activating factor and recovery of the latter in the vacuole fraction. *J. Biol. Chem.* **248**:1451-1458.
  54. Cabib, E., and R. E. Ulane. 1973. Chitin synthetase activating factor from yeast, a protease. *Biochem. Biophys. Res. Commun.* **50**:186-191.
  55. Camargo, E. P., C. P. Dietrich, D. Sonnenborn, and J. L. Strominger. 1971. Biosynthesis of chitin in spores and growing cells of *Blastocladiella emersonii*. *J. Biol. Chem.* **242**:3121-3128.
  56. Carbonell, L. M., and J. Rodriguez. 1968. Mycelial phase of *Paracoccidioides brasiliensis* and *Blastomyces dermatitidis*: an electron microscope study. *J. Bacteriol.* **96**:533-543.
  57. Cartledge, T. G., and D. X. Lloyd. 1972. Subcellular fractionation of particles containing acid hydrolases from *Saccharomyces carlsbergensis*. *Biochem. J.* **126**:755-757.
  58. Cassone, A. 1973. Improved visualization of wall ultrastructure in *Saccharomyces cerevisiae*. *Experientia* **29**:1303-1305.
  59. Chen, W. W., and W. J. Lennarz. 1977. Metabolism of lipid-linked N-acetylglucosamine intermediates. *J. Biol. Chem.* **252**:3473-3479.
  60. Corina, D. L., and K. A. Munday. 1971. The metabolic stability of carbohydrates in the walls of hyphae of *Aspergillus clavatus*. *J. Gen. Microbiol.* **65**:253-257.
  61. Cortat, M., P. Matile, and F. Kopp. 1973. Intracellular localization of mannan synthetase activity in budding baker's yeast. *Biochem. Biophys. Res. Commun.* **53**:482-489.
  62. Cortat, M., P. Matile, and A. Wiemken. 1972. Isolation of glucanase-containing vesicles from budding yeast. *Arch. Microbiol.* **82**:189-205.
  63. De Rousset-Hall, A., and G. W. Gooday. 1975. A kinetic study of solubilized chitin synthetase preparation from *Coprinus cinereus*. *J. Gen. Microbiol.* **89**:146-154.
  64. De Vries, O. M. H., and J. G. H. Wessels. 1975. Chemical analysis of cell wall regeneration and reversion of protoplasts from *Schizophyllum commune*. *Arch. Microbiol.* **102**:209-218.
  65. Djaczenko, W., and A. Cassone. 1972. Visualization of new ultrastructural components in the cell wall of *Candida albicans* with fixatives containing TAPO. *J. Cell Biol.* **52**:186-190.

66. Dominguez, A., J. R. Villanueva, and R. Sentandreu. 1978. Inositol deficiency in *Saccharomyces cerevisiae* NCYC 86. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 44:25-34.
67. Dominguez, A., J. R. Villanueva, and R. Sentandreu. 1978. Biosynthesis of *Saccharomyces cerevisiae* glycoproteins: nature of some participating glycolipids. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 44:183-192.
68. Dubé, J., G. Setterfield, G. Kiss, and C. V. Lusena. 1973. Fate of plasma membrane of *Saccharomyces cerevisiae* during cell rupture. *Can. J. Microbiol.* 19:285-290.
69. Durán, A., B. Bowers, and E. Cabib. 1975. Chitin synthetase zymogen is attached to yeast plasma membrane. *Proc. Natl. Acad. Sci. U.S.A.* 72:3952-3955.
70. Durán, A., and E. Cabib. 1978. Solubilization and partial purification of yeast chitin synthase. Confirmation of the zymogenic nature of the enzyme. *J. Biol. Chem.* 253:4419-4425.
71. Eddy, A. A., and J. S. Woodhead. 1968. An alkali-soluble glucan fraction from the cell walls of the yeast *Saccharomyces carlsbergensis*. *FEBS Lett.* 1:67-68.
72. Eiserling, F. A., and R. C. Dickson. 1972. Assembly of viruses. *Annu. Rev. Biochem.* 41:467-502.
73. Elorza, M. V., C. M. Lostau, J. R. Villanueva, and R. Sentandreu. 1976. Cell wall synthesis regulation in *Saccharomyces cerevisiae*. Effect of RNA and protein inhibition. *Biochim. Biophys. Acta.* 454:263-272.
74. Elorza, M. V., and R. Sentandreu. 1969. Effect of cycloheximide on yeast cell wall synthesis. *Biochem. Biophys. Res. Commun.* 36:741-747.
75. Elorza, M. V., and R. Sentandreu. 1973. The effect of cycloheximide on mannosyl transferase activity of a membrane preparation from *Saccharomyces cerevisiae*, p. 205-209. In J. R. Villanueva, I. García-Acha, S. Gascón, and F. Uruburu (ed.), *Yeast, mould and plant protoplasts*. Academic Press Inc., New York and London.
76. Endo, A., K. Kakiki, and T. Misato. 1970. Mechanism of action of the antifungal agent polyoxin D. *J. Bacteriol.* 104:189-196.
77. Endo, A., and T. Misato. 1969. Polyoxin D, a competitive inhibitor of UDP-N-acetylglucosamine:chitin N-acetylglucosaminyltransferase in *Neurospora crassa*. *Biochem. Biophys. Res. Commun.* 37:718-722.
78. Eylar, E. H. 1966. On the biological role of glycoproteins. *J. Theor. Biol.* 10:89-113.
79. Farkaš, V., J. Kovarik, A. Košinová, and Š. Bauer. 1974. Autoradiographic study of mannan incorporation into the growing cell walls of *Saccharomyces cerevisiae*. *J. Bacteriol.* 117:265-269.
80. Farkaš, V., A. Svoboda, and Š. Bauer. 1970. Secretion of cell wall glycoproteins by yeast protoplasts. The effect of 2-deoxy-D-glucose and cycloheximide. *Biochem. J.* 118:755-758.
81. Farkaš, V., V. M. Vagabov, and Š. Bauer. 1976. Biosynthesis of yeast mannan. Diversity of mannosyltransferases in the mannan-synthesizing enzyme system from yeast. *Biochim. Biophys. Acta* 428:573-582.
82. Farkaš, V., V. M. Vagabov, and Š. Bauer. 1976. Biosynthesis of yeast mannan. Independent formation of two carbohydrate moieties. *Biochim. Biophys. Acta* 428:583-590.
83. Fèvre, M. 1977. Subcellular localization of glucanase and cellulase in *Saprolegnia monoica* Pringsheim. *J. Gen. Microbiol.* 103:287-295.
84. Fèvre, M., and C. Dumas. 1977.  $\beta$ -Glucan synthetases from *Saprolegnia monoica*. *J. Gen. Microbiol.* 103:297-306.
85. Fleet, G. H., and H. J. Phaff. 1974. Glucanases in *Schizosaccharomyces*. Isolation and properties of the cell wall associated  $\beta$ -(1-3)-glucanases. *J. Biol. Chem.* 249:1717-1928.
86. Fultz, S. A., and R. A. Wolf. 1972. Surface structure of *Allomyces* during germination and growth. *Mycologia* 64:212-218.
87. Furuya, A., and Y. Ikeda. 1966. Cell-wall lytic enzymes. II. Lytic enzyme of *Streptomyces* No 62. *J. Gen. Appl. Microbiol.* 6:49-60.
88. Gabriel, M. 1968. Formation and regeneration of protoplasts in the mould *Rhizopus nigricans*. *Folia Microbiol. (Prague)* 13:231-235.
89. Galun, E. 1972. Morphogenesis of *Trichoderma*: autoradiography of intact colonies labelled by (<sup>3</sup>H)N-acetylglucosamine as a marker of new cell wall biosynthesis. *Arch. Mikrobiol.* 86:305-314.
90. Galun, M., A. Braun, A. Frensdorff, and E. Galun. 1976. Hyphal walls of isolated lichen fungi. Autoradiographic localization of precursor incorporation and binding of fluorescein-conjugated lectins. *Arch. Microbiol.* 108:9-16.
91. Gander, J. E. 1974. Fungal cell wall glycoproteins and peptido-polysaccharides. *Annu. Rev. Microbiol.* 28:103-119.
92. Gander, J. E., L. R. Drewes, F. Fang, and A. Lui. 1977. 5-O- $\beta$ -D-galactofuranosyl-containing exocellular glycopeptide of *Penicillium charlesii*. Incorporation of mannose from GDP-D-mannose into glycopeptide. *J. Biol. Chem.* 252:2187-2193.
93. Gander, J. E., and F. Fang. 1977. Properties of *Penicillium* GDP-mannose:glycopeptide mannosyl transferase solubilized with Triton X-100. *J. Supramol. Struct.* 6:579-589.
94. García-Acha, I., F. López-Belmonte, and J. R. Villanueva. 1966. Regeneration of mycelial protoplasts of *Fusarium culmorum*. *J. Gen. Microbiol.* 45:515-523.
95. García Mendoza, C., and M. Novaes Ledieu. 1968. Chitin in the new wall of regenerating protoplasts of *Candida utilis*. *Nature (London)* 220:1035.
96. Gibson, R. K., C. E. Buckley, and J. F. Peberdy. 1976. Wall ultrastructure in regenerating protoplasts of *Aspergillus nidulans*. *Protoplasma* 89:381-387.
97. Girbardt, M. 1969. Die Ultrastruktur der Apikalregion von Pilzhypphen. *Protoplasma* 67:413-441.
98. Glaser, L., and D. H. Brown. 1957. The syn-

- thesis of chitin in cell free extracts of *Neurospora crassa*. J. Biol. Chem. **228**:729-742.
99. Gold, M. H., and H. J. Hahn. 1976. Role of a mannosyl lipid intermediate in the synthesis of *Neurospora crassa* glycoproteins. Biochemistry **15**:1808-1813.
  100. Gooday, G. W. 1971. An autoradiographic study of hyphal growth of some fungi. J. Gen. Microbiol. **67**:125-133.
  101. Gooday, G. W. 1977. Biosynthesis of fungal wall-mechanisms and implications. J. Gen. Microbiol. **99**:1-11.
  102. Gooday, G. W., and A. De Rousset-Hall. 1975. Properties of chitin synthetase from *Coprinus cinereus*. J. Gen. Microbiol. **89**:137-145.
  103. Gooday, G. W., A. de Rousset-Hall, and D. Hunsley. 1976. Effect of polyoxin D on chitin synthesis in *Coprinus cinereus*. Trans. Br. Mycol. Soc. **67**:193-200.
  104. Grove, S. N., C. E. Bracker, and D. J. Morré. 1970. An ultrastructural basis for hyphal tip growth in *Pythium ultimum*. Am. J. Botany. **67**:245-266.
  105. Gunja-Smith, Z., N. B. Patil, and E. E. Smith. 1977. Two pools of glycogen in *Saccharomyces*. J. Bacteriol. **130**:818-825.
  106. Gunja-Smith, Z., and E. E. Smith. 1974. Evidence for periplasmic location of glycogen in *Saccharomyces*. Biochem. Biophys. Res. Commun. **56**:588-592.
  107. Hasilik, A. 1974. Inactivation of chitin synthase in *Saccharomyces cerevisiae*. Arch. Microbiol. **101**:295-301.
  108. Hasilik, A., and H. Holzer. 1973. Participation of the tryptophan synthase inactivating system from yeast in the activation of chitin synthase. Biochem. Biophys. Res. Commun. **53**:552-559.
  109. Hata, T., R. Hayashi, and E. Doi. 1967. Purification of yeast proteinases. I. Fractionation and some properties of the proteinases. Agric. Biol. Chem. **31**:150-159.
  110. Hayashibe, M., N. Abe, and M. Matsui. 1977. Mode of increase in cell wall polysaccharides in synchronously growing *Saccharomyces cerevisiae*. Arch. Microbiol. **114**:91-92.
  111. Hayashibe, M., and S. Katohda. 1973. Initiation of budding and chitin ring. J. Gen. Appl. Microbiol. **19**:23-39.
  112. Hayashibe, M., N. Sando, and S. Katohda. 1972. Cell wall formation in growing *Saccharomyces cerevisiae*, p. 811-817. In Proceedings of the Fourth International Fermentation Symposium. Fermentation Technology Today.
  113. Hills, G. J. 1973. Cell wall assembly *in vitro* from *Chlamydomonas reinhardtii*. Planta **115**:17-23.
  114. Holley, R. A., and D. K. Kidby. 1973. Role of vacuoles and vesicles in extracellular enzyme secretion from yeast. Can. J. Microbiol. **19**:113-117.
  115. Hori, M., K. Kakiki, and T. Misato. 1974. Interaction between polyoxin and active center of chitin synthetase. Agric. Biol. Chem. **38**:699-705.
  116. Houwik, A. L., and D. R. Kreger. 1953. The cell wall of yeasts—electron microscope and X-ray diffraction study. Antonie van Leeuwenhoek J. Microbiol. Serol. **19**:1-24.
  117. Hsu, A.-F., J. W. Baynes, and E. C. Heath. 1974. The role of dolichol oligosaccharide as an intermediate in glycoprotein biosynthesis. Proc. Natl. Acad. Sci. U.S.A. **71**:2391-2395.
  118. Hunsley, D. 1973. Apical wall structure in hyphae of *Phytophthora parasitica*. New Phytol. **72**:985-990.
  119. Hunsley, D., and J. H. Burnett. 1970. The ultrastructural architecture of the walls of some hyphal fungi. J. Gen. Microbiol. **62**:203-218.
  120. Hunsley, D., and G. W. Gooday. 1974. The structure and development of septa in *Neurospora crassa*. Protoplasma **82**:125-146.
  121. Hunsley, D., and D. Kay. 1976. Wall structure of *Neurospora* hyphal apex—immunofluorescent localization of wall surface antigens. J. Gen. Microbiol. **95**:233-248.
  122. Isaac, S., N. S. Ryder, and J. F. Peberdy. 1978. Distribution and activation of chitin synthase in protoplast fractions released during the lytic digestion of *Aspergillus nidulans* hyphae. J. Gen. Microbiol. **105**:45-50.
  123. Jan, Y. N. 1974. Properties and cellular localization of chitin synthetase in *Phycomyces blakesleanus*. J. Biol. Chem. **249**:1973-1979.
  124. Jaspers, H. T. A., K. Christiansen, and J. van Stevenick. 1975. An improved method for the preparation of yeast enzymes *in situ*. Biochem. Biophys. Res. Commun. **65**:1434-1439.
  125. Johnson, B. F. 1968. Lysis of yeast cell walls induced by 2-deoxyglucose at sites of glucan synthesis. J. Bacteriol. **95**:1169-1172.
  126. Jones, E. W. 1977. Proteinase mutants of *Saccharomyces cerevisiae*. Genetics **85**:23-33.
  127. Jung, P., and W. Tanner. 1973. Identification of the lipid intermediate in yeast mannan biosynthesis. Eur. J. Biochem. **37**:1-6.
  128. Kanetsuna, F., L. M. Carbonell, I. Azuma, and Y. Yamamura. 1972. Biochemical studies on the thermal dimorphism of *Paracoccidioides brasiliensis*. J. Bacteriol. **110**:208-218.
  129. Karson, E., and C. E. Ballou. 1978. Biosynthesis of yeast mannan. Properties of a mannosylphosphate transferase in *Saccharomyces cerevisiae*. J. Biol. Chem. **253**:6484-6492.
  130. Katoh, Y., A. Kuninaka, H. Yoshino, A. Takatsuki, M. Yamasaki, and G. Tamura. 1976. Formation of fungal multinuclear giant cells by tunicamycin. J. Gen. Appl. Microbiol. **22**:247-258.
  131. Katz, D., and R. F. Rosenberger. 1971. Hyphal wall synthesis in *Aspergillus nidulans*: effect of protein synthesis inhibition and osmotic shock on chitin insertion and morphogenesis. J. Bacteriol. **108**:184-190.
  132. Katz, D., and R. F. Rosenberger. 1971. Lysis of an *Aspergillus nidulans* mutant blocked in chitin synthesis and its relation to wall assembly and wall metabolism. Arch. Mikrobiol. **80**:284-292.
  133. Keller, F. A., and E. Cabib. 1971. Chitin and

- yeast budding: properties of chitin synthetase from *Saccharomyces carlsbergensis*. *J. Biol. Chem.* **246**:160-166.
134. Kidby, D. K., and R. Davies. 1970. Invertase and disulphide bridges in the yeast wall. *J. Gen. Microbiol.* **61**:327-333.
  135. Kopecká, M., H. J. Phaff, and G. H. Fleet. 1974. Demonstration of a fibrillar component in the cell wall of the yeast *Saccharomyces cerevisiae* and its chemical nature. *J. Cell Biol.* **62**:66-76.
  136. Košinová, A., V. Farkaš, S. Machala, and Š. Bauer. 1974. Site of mannan synthesis in yeast. An autoradiographic study. *Arch. Microbiol.* **99**:255-263.
  137. Kotyk, A., and K. Janáček. 1977. Membrane transport. An interdisciplinary approach, p. 108-109. Plenum Publishing Corp., New York.
  138. Kozak, L. P., and R. K. Bretthauer. 1970. Studies on the biosynthesis of *Hansenula holstii* mannans from guanosine diphosphate mannose. *Biochemistry* **9**:1115-1122.
  139. Krátky, Z., P. Biely, and Š. Bauer. 1975. Mechanism of 2-deoxy-D-glucose inhibition of cell wall polysaccharide and glycoprotein biosynthesis in *Saccharomyces cerevisiae*. *Eur. J. Biochem.* **54**:459-467.
  140. Krátky, Z., P. Biely, and Š. Bauer. 1975. Wall mannan of *Saccharomyces cerevisiae*. Metabolic stability and release into growth medium. *Biochim. Biophys. Acta* **404**:1-6.
  141. Kuo, S.-C., and J. O. Lampen. 1972. Inhibition by 2-deoxy-D-glucose of synthesis of glycoprotein enzymes by protoplasts of *Saccharomyces*: relation to inhibition of sugar uptake and metabolism. *J. Bacteriol.* **111**:419-429.
  142. Kuo, S.-C., and J. O. Lampen. 1974. Tunicamycin—an inhibitor of yeast glycoprotein synthesis. *Biochem. Biophys. Res. Commun.* **58**:287-295.
  143. Kuo, S.-C., and J. O. Lampen. 1976. Tunicamycin inhibition of (<sup>3</sup>H)glucosamine incorporation into yeast glycoproteins: binding of tunicamycin and interaction with phospholipids. *Arch. Biochem. Biophys.* **172**:574-581.
  144. Kurland, C. G. 1972. Structure and function of bacterial ribosome. *Annu. Rev. Biochem.* **41**:377-408.
  145. Kushner, D. J. 1969. Self-assembly of biological structures. *Bacteriol. Rev.* **33**:302-345.
  - 145a. Lahoz, R. 1967. Quantitative changes in the content of nonnitrogenous compounds during autolysis of *Aspergillus terreus*. *J. Gen. Microbiol.* **46**:451-456.
  146. Lampen, J. O. 1968. External enzymes of yeast: their nature and formation. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **34**:1-18.
  147. Larriba, G., M. V. Elorza, J. R. Villanueva, and R. Sentendreu. 1976. Participation of dolichol phosphomannose in the glycosylation of yeast wall mannoproteins at the polysomal level. *FEBS Lett.* **71**:316-320.
  148. Lehle, L., F. Bauer, and W. Tanner. 1977. The formation of glycosidic bonds in yeast glycoproteins. Intracellular localisation of the reactions. *Arch. Microbiol.* **114**:77-81.
  149. Lehle, L., F. Fartaczek, W. Tanner, and H. Kauss. 1976. Formation of polyprenol-linked mono- and oligosaccharides in *Phasoleus aureus*. *Arch. Biochem. Biophys.* **175**:419-426.
  150. Lehle, L., and R. T. Schwarz. 1976. Formation of dolichol monophosphate 2-deoxy-D-glucose and its interference with the glycosylation of mannoproteins in yeast. *Eur. J. Biochem.* **67**:239-245.
  151. Lehle, L., and W. Tanner. 1974. Membrane-bound mannosyl transferase in yeast glycoprotein biosynthesis. *Biochim. Biophys. Acta* **350**:225-235.
  152. Lehle, L., and W. Tanner. 1975. Formation of lipid-bound oligosaccharides in yeast. *Biochim. Biophys. Acta* **399**:364-374.
  153. Lehle, L., and W. Tanner. 1976. The specific site of tunicamycin inhibition in the formation of dolichol-bound N-acetylglucosamine derivatives. *FEBS Lett.* **71**:167-170.
  154. Lehle, L., and W. Tanner. 1978. Biosynthesis and characterization of large dolichylphosphate-linked oligosaccharides in *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* **539**:218-229.
  155. Lehle, L., and W. Tanner. 1978. Glycosyl transfer from dolichyl-phosphate sugars to endogenous and exogenous glycoprotein acceptors in yeast. *Eur. J. Biochem.* **83**:563-570.
  156. Leloir, L. F. 1971. Two decades of research on the biosynthesis of saccharides. *Science* **172**:1299-1303.
  157. Lennarz, W. J. 1975. Lipid linked sugars in glycoprotein synthesis. *Science* **188**:986-991.
  158. Létoublon, R. C. P., J. Combe, and R. Got. 1973. Transfert du mannose, à partir de GDP-mannose à des accepteurs lipidiques chez *Aspergillus niger*. *Eur. J. Biochem.* **40**:95-101.
  159. Létoublon, R., and R. Got. 1974. Rôle d'un intermédiaire dans le transfert du mannose à des accepteurs glycoprotéiques endogènes chez *Aspergillus niger*. *FEBS Lett.* **46**:214-217.
  160. Létoublon, R., M. Richard, P. Louisot, and R. Got. 1971. Étude du transfert enzymatique de D-(<sup>14</sup>C)mannose à partir de GDP-(<sup>14</sup>C)mannose, sur un accepteur endogène dans les microsomes d'*Aspergillus niger*. *Eur. J. Biochem.* **18**:194-200.
  161. Linnemans, W. A. M., P. Boer, and P. F. Elbers. 1977. Localization of acid phosphatase in *Saccharomyces cerevisiae*: a clue to cell wall formation. *J. Bacteriol.* **131**:638-644.
  162. Liras, P., and S. Gascón. 1971. Biosynthesis and secretion of yeast invertase. Effect of cycloheximide and 2-deoxy-D-glucose. *Eur. J. Biochem.* **23**:160-165.
  163. López-Romero, E., S. Bartnicki-Garcia, and J. Ruiz-Herrera. 1978. Purification of inhibitory protein of chitin synthetase from *Mucor rouxii*. *Biochim. Biophys. Acta* **525**:338-345.
  164. López-Romero, E., and J. Ruiz-Herrera. 1976. Synthesis of chitin by particulate preparations from *Aspergillus flavus*. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **42**:261-276.

165. López-Romero, E., and J. Ruiz-Herrera. 1977. Biosynthesis of  $\beta$ -glucans by cell free extracts from *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta.* **500**:372-384.
166. Lovett, J. S. 1968. Reactivation of ribonucleic acid and protein synthesis during germination of *Blastocladiella* zoospores and the role of the ribosomal nuclear cap. *J. Bacteriol.* **96**: 962-969.
167. Lyons, T. P., and J. S. Hough. 1970. Role of yeast cell walls in brewing. *Brew. Dig.* **45**:52-60.
168. Mahadevan, P. R., and U. R. Mahadkar. 1970. Role of enzymes in growth and morphology of *Neurospora crassa*: cell-wall-bound enzymes and their possible role in branching. *J. Bacteriol.* **101**:941-947.
169. Manners, D. J., and A. J. Masson. 1969. The structure of two glucans from yeast cell walls. *FEBS Lett.* **4**:122-124.
170. Manners, D. J., A. J. Masson, and J. C. Patterson. 1973. The structure of a  $\beta$ -(1,3)-D-glucan from yeast cell walls. *Biochem. J.* **135**:19-30.
171. Manners, D. J., A. J. Masson, J. C. Patterson, H. Björndal, and B. Lindberg. 1973. The structure of a  $\beta$ -(1,6)-D-glucan from yeast cell walls. *Biochem. J.* **135**:31-36.
172. Marchant, R. 1966. Wall structure and spore germination in *Fusarium culmorum*. *Ann. Bot. (London)* **30**:821-830.
173. Marchant, R., and D. G. Smith. 1968. A serological investigation of hyphal growth in *Fusarium culmorum*. *Arch. Mikrobiol.* **63**:85-94.
174. Mariott, M. S. 1977. Mannan-protein location and biosynthesis in plasma membranes from the yeast form of *Candida albicans*. *J. Gen. Microbiol.* **103**:51-59.
175. Marshall, R. D. 1972. Glycoproteins. *Annu. Rev. Biochem.* **41**:673-702.
176. Matile, P., M. Cortat, A. Wiemken, and A. Frey-Wysling. 1971. Isolation of glucanase-containing particles from budding *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A.* **68**:636-640.
177. Matsumae, A., and E. C. Cantino. 1971. Sensitivity of the spores of *Blastocladiella emersonii* and related fungi to antibiotics and some other drugs. *J. Antibiot.* **24**:77-84.
178. Mayer, R., R. W. Parish, and H. R. Hohl. 1976. Hyphal tip growth in *Phytophthora*. Gradient distribution and ultrahistochemistry of enzymes. *Arch. Microbiol.* **110**:215-224.
179. Mayer, R. M. 1971. The enzymatic synthesis of the phosphomannan of *Hansenula capsulata*. *Biochim. Biophys. Acta* **252**:39-47.
180. McLellan, W. L., Jr., and J. O. Lampen. 1968. Phosphomannanase (PR-factor), an enzyme required for the formation of yeast protoplasts. *J. Bacteriol.* **95**:967-974.
181. McMurrough, I., and S. Bartnicki-Garcia. 1971. Properties of a particulate chitin synthetase from *Mucor rouxii*. *J. Biol. Chem.* **246**: 4008-4016.
182. McMurrough, I., and Bartnicki-Garcia. 1973. Inhibition and activation of chitin synthesis by *Mucor rouxii* cell extracts. *Arch. Biochem. Biophys.* **158**:812-816.
183. McMurrough, I., A. Flores-Carreón, and S. Bartnicki-Garcia. 1971. Pathway of chitin synthesis and cellular localization of chitin synthetase in *Mucor rouxii*. *J. Biol. Chem.* **246**: 3999-4007.
184. Meyer, J., and P. Matile. 1975. Subcellular distribution of yeast invertase isoenzymes. *Arch. Microbiol.* **103**:51-55.
185. Michalenko, G. O., H. L. Hohl, and D. Rast. 1976. Chemistry and architecture of the mycelial wall of *Agaricus bisporus*. *J. Gen. Microbiol.* **92**:251-262.
186. Mishra, N. C., and E. L. Tatum. 1972. Effect of L-sorbose on polysaccharide synthetases of *Neurospora crassa*. *Proc. Natl. Acad. Sci. U.S.A.* **69**:313-317.
187. Moor, H. 1967. Endoplasmic reticulum as the initiator of bud formation in yeast. *Arch. Mikrobiol.* **57**:135-146.
188. Moore, P. M., and J. F. Peberdy. 1976. A particulate chitin synthetase from *Aspergillus flavus* Link: the properties, location and the levels of activity in mycelium and regenerating protoplast preparations. *Can. J. Microbiol.* **22**: 915-921.
189. Moreno, F., A. G. Ochoa, S. Gascón, and J. R. Villanueva. 1975. Molecular forms of yeast invertase. *Eur. J. Biochem.* **50**:571-579.
190. Nakajima, T., and C. E. Ballou. 1974. Characterization of the carbohydrate fragments obtained from *Saccharomyces cerevisiae* mannan by alkaline degradation. *J. Biol. Chem.* **249**:7679-7684.
191. Nakajima, T., and C. E. Ballou. 1974. Structure of the linkage region between the polysaccharide and protein parts of *Saccharomyces cerevisiae* mannan. *J. Biol. Chem.* **249**:7685-7694.
192. Nakajima, T., and C. E. Ballou. 1975. Microheterogeneity of the inner core region of yeast mannoprotein. *Biochem. Biophys. Res. Commun.* **66**:870-879.
193. Nakajima, T., and C. E. Ballou. 1975. Yeast manno-protein biosynthesis: solubilization and selective assay of four mannosyltransferases. *Proc. Natl. Acad. Sci. U.S.A.* **72**:3912-3916.
194. Nakayama, K., Y. Araki, and E. Ito. 1976. The formation of a mannose-containing trisaccharide on a lipid and its transfer to proteins in yeast. *FEBS Lett.* **72**:287-290.
195. Namba, H., and H. Kuroda. 1974. Studies on fungicides. X. Biosyntheses of  $\beta$ -glucan and chitin-like substance of cell wall from *Cochliobolus miyabeanus*. (1). *Chem. Pharm. Bull. (Japan)* **22**:610-616.
196. Namba, H., and H. Kuroda. 1974. Studies on fungicides. XII. Biosyntheses of  $\beta$ -glucan and chitin-like substance of cell wall from *Cochliobolus miyabeanus*. (2). *Chem. Pharm. Bull. (Japan)* **22**:1895-1901.
197. Nečas, O. 1965. Mechanism of regeneration of yeast protoplasts. III. Electron microscopy of growing protoplasts. *Folia Biol. (Prague)* **11**:

- 371-377.
198. Nečas, O. 1971. Cell wall synthesis in yeast protoplasts. *Bacteriol. Rev.* **35**:149-170.
  199. Nečas, O., A. Svoboda, and M. Kopecká. 1968. The effect of cycloheximide (actidione) on cell wall synthesis in yeast protoplasts. *Exp. Cell Res.* **53**:291-293.
  200. Northcote, D. H., and R. W. Horne. 1952. The chemical composition and structure of the yeast cell wall. *Biochem. J.* **51**:232-236.
  201. Ottolenghi, P. 1967. The uptake of bovine serum-albumin by a strain of *Saccharomyces* and its physico-pathological consequences. *C. R. Trav. Lab. Carsberg* **36**:95-111.
  202. Parodi, A. J. 1977. Synthesis of glucosyl-dolichol derivatives in yeast and their role in protein glycosylation. *Eur. J. Biochem.* **75**:171-180.
  203. Parodi, A. J. 1978. Lipid intermediates in the synthesis of inner core of yeast mannan. *Eur. J. Biochem.* **83**:253-259.
  204. Peberdy, J. F., and P. M. Moore. 1975. Chitin synthetase in *Mortierella vinacea*, properties, cellular location and synthesis in growing cultures. *J. Gen. Microbiol.* **90**:228-236.
  205. Phaff, H. J. 1971. Structure and biosynthesis of the yeast cell envelope, p. 135-210. In A. H. Rose and J. S. Harrison (ed.) *The yeasts*, vol. 2. Academic Press Inc., New York and London.
  206. Phaff, H. J. 1977. Enzymatic yeast cell wall degradation. *Adv. Chem. Ser.* **160**:244-282.
  207. Polacheck, Y., and R. F. Rosenberger. 1975. Autolytic enzymes in hyphae of *Aspergillus nidulans*: their action on old and newly formed walls. *J. Bacteriol.* **121**:332-337.
  208. Porter, C. A., and E. G. Jaworski. 1966. The synthesis of chitin by particulate preparations of *Allomyces macrogynus*. *Biochemistry* **5**:1149-1154.
  209. Potgieter, H. J., and M. Alexander. 1965. Polysaccharide components of *Neurospora crassa* hyphal walls. *Can. J. Microbiol.* **11**:122-125.
  210. Poulain, D., G. Tronchin, J. F. Dubremetz, and J. Biguet. 1978. Ultrastructure of the cell wall of *Candida albicans* blastospores: study of its constitutive layers by the use of a cytochemical technique revealing polysaccharides. *Ann. Microbiol. (Paris)* **129**:141-153.
  211. Raizada, M. K., H. G. Kloepper, J. S. Schutzbach, and H. Ankel. 1974. Biosynthesis of oligogalactosyl side chains of the cell envelope glycoprotein of *Cryptococcus laurentii*. *J. Biol. Chem.* **249**:6080-6086.
  212. Robertson, N. F. 1965. The fungal hypha. *Trans. Br. Mycol. Soc.* **48**:1-8.
  213. Romano, A. H. 1966. Dimorphism, p. 181-209. In G. C. Ainsworth and A. S. Sussman (ed.) *The fungi*, vol. 2. Academic Press Inc., New York and London.
  214. Roussel, I., I. Harrison, and G. Stewart. 1973. Studies on the formation of spheroplasts from stationary phase *Saccharomyces cerevisiae*. *J. Inst. Brew. London* **73**:48-54.
  215. Ruiz-Herrera, J., and S. Bartnicki-Garcia. 1974. Synthesis of cell wall microfibrils in vitro by a "soluble" chitin synthetase from *Mucor rouxii*. *Science* **186**:357-359.
  216. Ruiz-Herrera, J., E. López-Romero, and S. Bartnicki-Garcia. 1977. Properties of chitin synthetase in isolated chitosomes from yeast cells of *Mucor rouxii*. *J. Biol. Chem.* **252**:3338-3343.
  217. Ruiz-Herrera, J., and R. Sentandreu. 1975. Site of initial glycosylation of mannoproteins from *Saccharomyces cerevisiae*. *J. Bacteriol.* **124**:127-133.
  218. Ruiz-Herrera, J., V. O. Sing, V. J. van der Voude, and S. Bartnicki-Garcia. 1975. Microfibril assembly by granules of chitin synthetase. *Proc. Natl. Acad. Sci. U.S.A.* **72**:2706-2710.
  219. Ryder, N. S., and J. F. Peberdy. 1977. Chitin synthetase in *Aspergillus nidulans*: properties and proteolytic activation. *J. Gen. Microbiol.* **99**:69-76.
  220. Ryder, N. S., and J. F. Peberdy. 1977. Chitin synthase activity in dormant conidia of *Aspergillus nidulans*. *FEMS Microbiol. Lett.* **2**:199-201.
  221. Saheki, T., and H. Holzer. 1974. Comparisons of the tryptophan synthase inactivating enzymes with proteinases from yeast. *Eur. J. Biochem.* **42**:621-626.
  222. Scherrer, R., L. Loudon, and P. Gerhardt. 1974. Porosity of the yeast cell wall and membrane. *J. Bacteriol.* **118**:534-540.
  223. Schlenk, F., and J. L. Dainko. 1965. Action of ribonuclease preparations on viable yeast cells and spheroplasts. *J. Bacteriol.* **89**:428-436.
  224. Schmidt, R., and S. Jannsen. 1974. The effect of mannose on the synthesis of the glycoprotein acid phosphatase in a mannose-deficient mutant of *Schizosaccharomyces pombe*. *Biochim. Biophys. Acta* **362**:13-16.
  225. Schutzbach, J. S., and H. Ankel. 1969. Biosynthesis of oligosaccharide components of *Cryptococcus laurentii* cell wall. *FEBS Lett.* **5**:145-148.
  226. Schutzbach, J. S., and H. Ankel. 1971. Multiple mannosyl transferases in *Cryptococcus laurentii*. *J. Biol. Chem.* **246**:2187-2194.
  227. Schutzbach, J. S., M. K. Raizada, and H. Ankel. 1974. Heteroglycan synthesis in *Cryptococcus laurentii*. *J. Biol. Chem.* **249**:2953-2958.
  228. Schwarz, U., A. Asmus, and H. Frank. 1969. Autolytic enzymes and cell division in *Escherichia coli*. *J. Mol. Biol.* **41**:419-429.
  229. Seichertová, O., K. Beran, Z. Holan, and V. Pokorný. 1973. The chitin-glucan complex of *Saccharomyces cerevisiae*. II. Location of the complex in the encircling region of the bud scar. *Folia Microbiol. (Prague)* **18**:207-211.
  230. Selitrenikoff, C. P., D. Allin, and D. R. Sonneborn. 1976. Chitin biosynthesis during *Blasotradiella* zoospore germination: evidence that hexosamine biosynthetic pathway is post-translationally activated during cell differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **73**:534-538.

231. Sentandreu, R., M. V. Elorza, and J. R. Villanueva. 1975. Synthesis of yeast wall glucan. *J. Gen. Microbiol.* **90**:13-20.
232. Sentandreu, R., and J. O. Lampen. 1970. Biosynthesis of yeast mannan: inhibition of synthesis of mannose acceptor by cycloheximide. *FEBS Lett.* **11**:95-99.
233. Sentandreu, R., and J. O. Lampen. 1972. Biosynthesis of mannan in *Saccharomyces cerevisiae*. Isolation of a lipid intermediate and its identification as a mannosyl-1-phosphoryl polyphenol. *FEBS Lett.* **27**:331-334.
234. Sentandreu, R., and D. H. Northcote. 1968. The structure of a glycopeptide isolated from the yeast cell wall. *Biochem. J.* **109**:419-432.
235. Sentandreu, R., and D. H. Northcote. 1969. The characterization of oligosaccharides attached to threonine and serine in mannan glycopeptides obtained from the cell wall of yeast. *Carbohydr. Res.* **10**:584-585.
236. Sharma, C. B., P. Babcinski, L. Lehle, and W. Tanner. 1974. The role of dolichol monophosphate in glycoprotein biosynthesis in *Saccharomyces cerevisiae*. *Eur. J. Biochem.* **46**:35-41.
237. Shimoda, C., and N. Yanagishima. 1971. Role of cell wall-degrading enzymes in auxin-induced cell expansion in yeast. *Physiol. Plant.* **24**:46-50.
238. Sierra, J. M., R. Sentandreu, and J. R. Villanueva. 1973. Regulation of wall synthesis during *Saccharomyces cerevisiae* cell cycle. *FEBS Lett.* **34**:285-290.
239. Sipiczki, M., and V. Farkaš. 1979. Morphogenic effects of 2-deoxy-D-glucose on *Rhodospiridium toruloides*. *Folia Microbiol. (Prague)*, in press.
240. Sloat, B. F., and J. R. Pringle. 1978. A mutant of yeast defective in cellular morphogenesis. *Science* **200**:1171-1173.
241. Slyman, C. L., and C. W. Slyman. 1962. Measurements of membrane potentials in *Neurospora*. *Science* **136**:876-877.
242. Smith, J. E., and D. R. Berry. 1974. An introduction to the biochemistry of fungal development, p. 106-155. Academic Press Inc., New York and London.
243. Smith, W. L., T. Nakajima, and C. E. Ballou. 1975. Biosynthesis of yeast mannan. Isolation of *Kluyveromyces lactis* mannan mutants and a study of the incorporation of N-acetyl-D-glucosamine into the polysaccharide side chains. *J. Biol. Chem.* **250**:3426-3435.
244. Soll, D. R., R. Bromberg, and D. R. Sonnenborn. 1971. Zoospore germination in *Blastocladiella emersonii*: cell differentiation without protein synthesis. *Proc. Natl. Acad. Sci. U.S.A.* **68**:459-463.
245. Soumalainen, H., and T. Nurminen. 1973. Structure and function of the yeast cell envelope, p. 167-186. In J. R. Villanueva, I. García-Acha, S. Gascón, and F. Uruburu (ed.), *Yeast, mould and plant protoplasts*. Academic Press Inc., New York and London.
246. Sternlicht, E., D. Katz, and R. F. Rosenberger. 1973. Subapical wall synthesis and wall thickening induced by cycloheximide in hyphae of *Aspergillus nidulans*. *J. Bacteriol.* **114**:819-823.
247. Strunk, C. 1969. Licht und elektronenmikroskopische Untersuchungen an regenerierenden Protoplasten von *Polystictus versicolor*. *Z. Allg. Mikrobiol.* **9**:205-216.
248. Svihla, G., J. L. Dainko, and F. Schlenk. 1969. Ultraviolet micrography of penetration of exogenous cytochrome *c* into the yeast cell. *J. Bacteriol.* **100**:498-504.
249. Tanner, W. 1969. A lipid intermediate in mannan biosynthesis in yeast. *Biochem. Biophys. Res. Commun.* **35**:144-150.
250. Thieme, T. R., and C. E. Ballou. 1971. Nature of the phosphodiester linkage of the phosphomannan from the yeast *Kloeckera brevis*. *Biochemistry* **10**:4121-4129.
251. Tkacz, J. S., and J. O. Lampen. 1972. Wall replication in *Saccharomyces* species: use of fluorescein-conjugated concanavalin A to reveal the site of mannan insertion. *J. Gen. Microbiol.* **72**:243-247.
252. Touimi-Benjelloun, A., and R. Bonaly. 1976. Étude des parois de *Rhodotorula rubra*. VII. Influence du chloramphenicol sur la morphologie et les phosphatases des parois de *Rh. rubra*. *Chem. Biol. Interact.* **13**:403-414.
253. Trinci, A. P. J. 1978. Wall and hyphal growth. *Sci. Prog. London* **65**:75-99.
254. Trinci, A. P. J., and A. J. Coolinge. 1975. Hyphal wall growth in *Neurospora crassa* and *Geotrichum candidum*. *J. Gen. Microbiol.* **91**:355-361.
255. Troy, F. A., and H. Koffler. 1969. The chemistry and molecular architecture of *Penicillium chrysogenum*. *J. Biol. Chem.* **244**:5563-5576.
256. Tsiomenko, A. B., Yu. A. Shabalin, V. V. Dmitriev, V. M. Vagabov, B. A. Fikhte, and I. S. Kulaev. 1975. Biosynthesis of cell wall components by yeast protoplasts in the presence of cycloheximide and 2-deoxy-D-glucose (in Russian). *Izv. Akad. Nauk. SSSR* **225**:1446-1449.
257. Tsiomenko, A. B., V. M. Vagabov, J. Augustin, and I. S. Kulaev. 1974. About relationship of turnover of inorganic polyphosphates and mannan in yeast (in Russian). *Dokl. Akad. Nauk SSSR* **215**:478-480.
258. Ulane, R. E., and E. Cabib. 1974. The activating system of chitin synthetase from *Saccharomyces cerevisiae*. Purification and properties of an inhibitor of the activating factor. *J. Biol. Chem.* **249**:3418-3422.
259. Ulane, R. E., and E. Cabib. 1976. The activating system of chitin synthetase from *Saccharomyces cerevisiae*. Purification and properties of the activating factor. *J. Biol. Chem.* **251**:3367-3374.
260. Van Laere, A. J., and A. R. Carlier. 1978. Synthesis and proteolytic activation of chitin synthetase in *Phycomyces blakesleanus* Bur-

- geff. Arch. Microbiol. 116:181-184.
261. Van Rijn, H. J. M., P. Boer, and E. P. Steyn-Parvé. 1972. Biosynthesis of acid phosphatase of baker's yeast. Factors influencing its production by protoplasts and characterization of the secreted enzyme. Biochim. Biophys. Acta 268: 431-441.
262. Vessey, D. A., N. Lysenko, and D. Zakim. 1976. Evidence for multiple enzymes in the dolichol utilizing pathway of glycoprotein biosynthesis. Biochim. Biophys. Acta 428:138-145.
263. Villanueva, J. R. 1966. Protoplasts of fungi, p. 3-62. In G. C. Ainsworth and A. E. Sussman (ed.) The fungi, vol. 2. Academic Press Inc., New York and London.
264. Villanueva, J. R., M. Gacto, and J. Sierra. 1973. Enzymic composition of a lytic system from *Micromonospora chalybeata* AS, p. 3-24. In J. R. Villanueva, I. García-Acha, S. Gascón, and F. Uruburu (ed.), Yeast, mould and plant protoplasts. Academic Press Inc., New York and London.
265. Villanueva, J. R., and I. García-Acha. 1972. The phenomenon of regeneration in fungal protoplasts. Rev. Latinoam. Microbiol. 14:49-64.
266. Voříšek, J., J. Ludvík, and Z. Řeháček. 1974. Cytochemical localization of polysaccharides in *Claviceps paspali* ultrastructure during submerged formation of alkaloids. J. Bacteriol. 118:285-294.
267. Voříšek, J., and V. Pokorný. 1975. Cytochemical detection of polysaccharides on the surface of the cell membrane complex in fungi. Arch. Microbiol. 102:293-298.
268. Vršanská, M., P. Biely, and Z. Krátky. 1977. Enzymes of the yeast lytic system produced by *Arthrobacter* GJM-1 bacterium and their role in the lysis of yeast cell walls. Z. Allg. Mikrobiol. 17:465-480.
269. Vršanská, M., Z. Krátky and P. Biely. 1977. Lysis of intact yeast cells and isolated cell walls by an inducible enzyme system of *Arthrobacter* GJM-1. Z. Allg. Mikrobiol. 17:391-402.
270. Wang, M. C., and S. Bartnicki-Garcia. 1966. Biosynthesis of  $\beta$ -1,3 and  $\beta$ -1,6 linked glucan by *Phytophthora cinnamomi* hyphal walls. Biochem. Biophys. Res. Commun. 24:832-837.
271. Wang, M. C., and S. Bartnicki-Garcia. 1970. Structure and composition of walls of the yeast form of *Verticillium albo-atrum*. J. Gen. Microbiol. 64:41-54.
272. Wang, M. C., and S. Bartnicki-Garcia. 1976. Synthesis of  $\beta$ -1,3-glucan microfibrils by a cell-free extract from *Phytophthora cinnamomi*. Arch. Biochem. Biophys. 175:351-354.
273. Wick, A. N., D. R. Drury, H. I. Nakada, and J. B. Wolfe. 1957. Localization of the primary metabolic block produced by 2-deoxy-glucose. J. Biol. Chem. 224:963-969.
274. Wong, W., F. E. Young, and A. N. Chatterjee. 1975. Regulation of bacterial cell walls: turnover of cell wall in *Staphylococcus aureus*. J. Bacteriol. 120:837-843.
275. Yen, P. H., and C. E. Ballou. 1973. Composition of a specific intercellular agglutination factor. J. Biol. Chem. 248:8316-8318.
276. Yphantis, D. A., J. L. Dainko, and F. Schlenk. 1967. Effect of some proteins on the yeast cell membrane. J. Bacteriol. 94:1509-1515.
277. Zevenhuizen, L. P. T. M., and S. Barnicki-Garcia. 1969. Chemical structure of the insoluble hyphal wall glucan of *Phytophthora cinnamomi*. Biochemistry 8:1496-1500.
278. Zonneveld, B. J. M. 1972. Morphogenesis in *Aspergillus nidulans*. The significance of the cell wall and  $\alpha$ -1,3-glucanase for cleistothecium development. Biochim. Biophys. Acta 273: 174-187.